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Study of the Alkaline Nitrobenzene Oxidation of Chlorite Lignin

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A STUDY OF THE ALKALINE NITROBENZENE OXIDATION OF CHLORITE LIGNIN

A thesis submitted by

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INTRODUCTION

The action of acidic solutions of sodium chlorite on wood has received considerable attention in recent years not only because of its importance as a bleaching operation on a commercial scale but also for its use in the laboratory as a means of preparing holocellulose. In several instances investigators have succeeded in isolating from the reaction a new lignin preparation which has been called chlorite lignin.

Chlorite lignin has been characterized only insofar as elemental composition and, to some degree, the ratios and types of functional groups are concerned. No extended effort has yet been made to investigate the nature of the more fundamental units which make up the structure of chlorite lignin.

It was felt that an alkaline nitrobenzene oxidation constituted the best approach to the solution of this problem. Many investigators have shown that identification of the fragments produced in this way from the degradative oxidation of other lignin preparations gave considerable insight into the composition of the starting material. The recent advent of chromatographic techniques as an aid in the separation of complex mixtures of organic compounds was expected to aid materially in the work.

The primary goal of this investigation was an elucidation of the structure of chlorite lignin itself, but, as is the ultimate aim of all work in the lignin field, it was hoped that whatever knowledge was gained might help achieve a greater understanding of the lignin complex itself as it occurs in wood.

HISTORICAL REVIEW

In the past ten years there has been a number of investigations concerned with the action of acidic solutions of sodium chlorite on wood. On the one hand, the goal has been the isolation of a holocellulose which approaches the total carbohydrate fraction of the wood. Other investigators have examined the role of sodium chlorite as a bleaching agent. In either case, its usefulness depends upon its marked specificity for solubilizing lignin while leaving the cellulose largely intact.

Sohn and Reiff (1) were among the first to attempt a recovery of the material in chlorite liquors obtained from the digestion of wood with acidic solutions of sodium chlorite. They found that acidification of these liquors with strong acids produced a white flocculent precipitate which they believed to be hemicellulosic in nature.

Jayme and Hanke (2) undertook an investigation of the materials which were dissolved during the chloriting of sprucewood. The chlorite liquor was acidified, purified by dialysis, and concentrated to dryness; alternatively, the liquor was dialyzed without acidification. Up to 50% of the weight of the material removed from the wood was recovered. The products, representing from 2 to 13% of the original wood, contained from 8.6 to 9.8% of methoxyl, from 46.2 to 52.7% of carbon, from 4.8 to 5.6% of hydrogen, and from 1.4 to 4.5% of chlorine. Acid hydrolysis showed them to contain from 20 to 28% of reducing sugars, tentatively identified as glucose, and from 5 to 10% of aldonic acids.

It remained for Barton (3) to recover a material from the chlorite liquor which was free of carbohydrate material. Working with slash pine,

he was able to separate the precipitate obtained by acidification of the liquor into a lignin and a carbohydrate fraction. This was accomplished by repeated purification of the precipitate, using alternate solution in dioxane and precipitation into ether. This product, which he termed "chlorite lignin A", had the following analysis:

	Found	Calculated for $C_{26}H_{28}O_{14}Cl$
Carbon, %	51.59	52.00
Hydrogen, %	4.64	4.67
Chlorine, %	5.95	5.92
Oxygen (by difference), %	37.81	37.41

It was characterized by acetylation and methylation studies and assigned a unit weight of 600 in which the following functional groups could be found:

$$\begin{matrix} CH_3O \\ CH_3O \\ HO \\ HO \end{matrix}\Big[\ C_{22}H_{18}O_6\ \Big]\begin{matrix} COOH \\ COOH \\ Cl \end{matrix}$$

The hydroxyl groups were shown to be nonphenolic in nature.

The highest yield of chlorite lignin A which Barton obtained was 2.75% (based on the original wood); this accounted for 10% of the Klason lignin content of the original wood. When the acidified liquor from which chlorite lignin A had been removed was further concentrated or acidified, it yielded a product termed "chlorite lignin B"; its formation was accompanied by the evolution of heat and gases presumed to be chlorine and chlorine dioxide. The product was not thoroughly characterized but it had a significantly higher chlorine content than chlorite lignin A (11.25%).

The ultraviolet absorption spectra for chlorite lignins A and B were similar to those typically shown by lignin preparations, with the notable exception that a relatively smooth curve was found which did not show the inflection in the 260 to 280 mmu region.

Bublitz (4), working with black spruce, isolated a product similar to Barton's chlorite lignin A by electrodialyzing the concentrated chlorite liquor and treating the purified liquor with an excess of alcohol. The resulting precipitate was purified by solution in dioxane and precipitation into ether. The purified product had a methoxyl content of 12.5% and a chlorine content of 4.0%. It was recovered in 4.8% yield based on the original wood or 17.3% based on the Klason lignin content of the original wood.

Wacek and Schroth (5) have recently isolated an acidic material from a tropical wood and from spruce by acidification of the 24% potassium hydroxide extract of the chlorite holocellulose. Their chloriting procedure, a modification of Jayme's (6), was not greatly different from that used by Barton and by Bublitz, who patterned their techniques after the work of Wise, Murphy, and D'Addieco (7). Wacek and Schroth purified their product by precipitation from methanolic solution into dilute acid. Hydrolysis showed the absence of carbohydrate material. The substances obtained from spruce and from a tropical wood in 5.5 and 6.2% yields, respectively (based on the wood) are compared below with Barton's chlorite lignin A from slash pine.

	Wacek and Schroth, spruce	Wacek and Schroth, tropical	Barton
Methoxyl, %	12.4	16.5	10.35
Carboxyl, %	9.7	7.3	7.50
Chlorine, %	3.3	"slight"	5.95

The tropical wood substance of Wacek and Schroth was subjected to alkaline nitrobenzene oxidation and was reported to yield vanillin, syringaldehyde, and syringic acid; the ratio of acid to aldehyde was said to be considerably higher than that usually obtained from this treatment of wood or acid-isolated lignin. They concluded that the nucleus of the lignin building stone was present and that the material was to be attributed to lignin.

The use of oxidative degradation as a means of elucidating the structure of lignin preparations has received new attention in recent years. Many oxidizing agents cause almost complete destruction of the lignin molecule. In contrast, nitrobenzene under alkaline conditions has been shown to yield a considerable quantity of simple compounds in which the benzene nucleus and its substituent groups have survived the degradation.

The use of nitrobenzene for this purpose was first described in a patent applied for in 1937 by Schulz (8); using a temperature of 200°* for one hour he obtained up to 10% of vanillin from spent sulfite liquor solids (based on the weight of the solids). Freudenberg, Lautsch, and Engler (9) isolated up up to 25% of vanillin (based on the weight of lignin material) from isolated lignin, lignosulfonic acid, and from wood itself by using a temperature of 160° for three hours. Hibbert and coworkers (10) applied the technique to angiosperms and obtained vanillin and syringaldehyde in a combined yield of 40 to 45% (based on the weight of lignin).

*All temperatures are in degrees Centigrade.

Pearl and Lewis (11) studied the nitrobenzene oxidation of spent sulfite liquor from the standpoint of recovering the nitrobenzene reduction products and obtained vanillin in yields up to 25% (based on the lignin).

Wacek and Kratzl (12) extended the study of the nitrobenzene oxidation to model compounds. They concluded that the side chain of an otherwise unsubstituted benzene nucleus is not subject to major attack unless there is conjugation between a double bond and carbonyl group and that a free hydroxyl group para to the side chain greatly enhances the susceptibility of the side chain to oxidation to a formyl or carboxyl group.

Leopold (13) studied some of the variables in the nitrobenzene oxidation on a series of model compounds and concluded that the optimum condition for vanillin formation was two hours at 180°.

Leopold (14) used these oxidation conditions on Norway spruce; through the use of adsorption and paper partition chromatography, he claimed the identification of thirteen different oxidation products. The major products were vanillin and vanillic acid isolated in 27.5 and 4.8% yield, respectively (based on the lignin content of the wood).

The halogenation of lignin introduces the halogen in the 6-position of the benzene nucleus as has been shown by Lautsch and Piazolo (15), who isolated 8% of 6-bromovanillin by treating cuoxam spruce bromolignin with 10% potassium hydroxide and oxygen in the presence of cobaltic hydroxide. No 5-bromovanillin was found, although the free phenolic groups in lignin have a strong orienting influence to the ortho position and make substitution into a free 5-position very likely. 5-Chlorovanillin was prepared by Barton (16) by the reaction of sodium chlorite on vanillin itself. 5-Iodovanillin was produced in 10% yield

by Lautsch and Piazolo (15) from cuoxam spruce iodolignin but this preparation was made through the intermediate acetomercury cuoxam compound.

Pearl (17) isolated 6-chlorovanillin from that portion of the aldehyde fraction of the ether extract obtained from the chlorite liquor of a black spruce holocellulose digestion which was not volatile with steam.

PRESENTATION OF THE PROBLEM

Although there are numerous instances in the literature of alkaline nitrobenzene oxidations of lignin preparations, it would be difficult to anticipate the results to be expected from the application of the technique to chlorite lignin. Consequently, a series of exploratory oxidations was considered essential in order to ascertain the yield of simple compounds, the identity of the major products, the best separation techniques to be used and, in general, to familiarize the investigator with the operation.

This was followed by a large-scale oxidation based on the procedure suggested by the preliminary work. In this way, it was hoped that the identity of compounds found in smaller yields might be established.

As the work progressed, it became apparent that vanillin, 6-chloro-vanillin, and vanillic acid constituted the major products of the reaction and that it was desirable to know the behavior of each individual compound under the conditions of the oxidation.

Finally, several experiments were planned in which the time-temperature relationships of the oxidation were varied to determine their influence on the products of the reaction.

Originally, an investigation of other oxidizing agents, in addition to alkaline nitrobenzene, had been planned but it was abandoned when the complex nature of the problem became apparent.

EXPERIMENTAL SECTION

GENERAL

ANALYTICAL PROCEDURES

Methoxyl determinations were performed on a semimicro scale according to Institute Method 18. Carbon and hydrogen determinations were carried out according to Institute Method 706. Chlorine analyses were made on a gravimetric basis, using either sodium peroxide or oxygen as the oxidizing agent in a Parr bomb.

Prior to analysis, samples were dried in vacuo over phosphorus pentoxide in an Abderhalden pistol.

PURIFICATION OF SOLVENTS

Anhydrous ether and 1,4-dioxane were prepared by treating the technical products with sodium.

The petroleum ether used in this work, unless otherwise stated, was reagent grade with a boiling range of 60 to 110°.

STARTING MATERIALS

Two types of chlorite lignin and three compounds were used as starting materials in this study.

Barton's Chlorite Lignin

Chlorite lignin A was prepared by Barton (3) from slash pine in the following way.

Wood meal, extracted with alcohol-benzene, was digested with sodium chlorite and acetic acid for 4.5 hours under the conditions shown in Table I.

TABLE I

CHLORITING CONDITIONS

Extracted wood meal, g. (ovendry)	1800
Consistency of solution, %	6
Temperature, °C.	75-80
Length of cook, hrs.	4.5
Amount of $NaClO_2$ (based on wood), %	110
Rate of $NaClO_2$ addition, g./hr.	495
pH maintained by addition of acetic acid	4.5-4.6

The holocellulose was filtered and washed with water, yielding a liquor which was aerated and further acidified with sulfuric acid. The flocculent precipitate which formed was collected by filtration and triturated with dioxane. Dehydration and precipitation of the dioxane-soluble materials into ether yielded chlorite lignin A. Table II describes the product which Barton obtained from his "Cook 7".

TABLE II

CHLORITE LIGNINS USED AS STARTING MATERIALS

	Barton	Bublitz
Yield (based on wood), %	1.7	4.8
Yield (based on Klason lignin content of wood), %	6.1	17.3
Methoxyl, %	10.6	12.5
Chlorine, %	7.5	4.0

Bublitz' Chlorite Lignin

Chlorite lignin was prepared by Bublitz (4) from black spruce. The chloriting procedure approximated that used by Barton (see Table I) but the liquor treatment was modified.

The liquor and washings were concentrated and electrodialyzed. After further concentration, the purified liquor was treated with eight volumes of alcohol. The resulting finely divided precipitate was removed by filtration and washed with alcohol. The alcoholic filtrate and washings, further concentrated to a thick syrup, were triturated with dioxane; dehydration and precipitation of the dioxane-soluble materials into ether yielded the final chlorite lignin product.

A portion of the product was purified by successive suspensions in ether and low boiling petroleum ether. The purified product had a methoxyl content of 12.5% and a chlorine content of 4.0% (see Table II).

Vanillin

Monsanto vanillin U.S.P. was used in Oxidation 5.

6-Chlorovanillin

The 6-chlorovanillin used in Oxidation 6 was prepared from vanillin according to the procedure of Raiford and Lichty (18) and had a melting point of 168.5 to 169.5°*.

Vanillic Acid

The vanillic acid used in Oxidation 7 had been prepared from vanillin by oxidation with silver oxide (19). It was further purified by crystallization from dilute ethanol and had a melting point of 207 to 208°.

*All melting points were determined in a capillary and are uncorrected.

OXIDATION PROCEDURES

With the exception of Oxidation 4, all oxidations were carried out in a 420-ml. stainless steel bomb (26 mm. inside diameter and 80 cm. in length) fitted with a threaded plug containing a thermometer well and sealed with a lead or copper gasket. The bomb was placed within an electrically heated jacket which was rocked 36 times a minute through an angle of approximately 30°.

Oxidation 4 was carried out in a one-gallon autoclave equipped with a spade-type stirrer which rotated 36 times a minute. Heating was achieved through the use of a large gas burner placed under the autoclave.

The reactant ratios were arbitrarily based on those of Freudenberg and coworkers (9); for each 7.5 grams of material to be oxidized there were added 17.5 ml. of nitrobenzene and 300 ml. of 2 N sodium hydroxide.

Oxidations 1 through 7 were arbitrarily assigned the heating conditions used by Freudenberg and coworkers (9)--namely, 3 hours at 160°. Oxidation 8 was carried out under Leopold's conditions--2 hours at 180° (13). Oxidation 9 was again kept at 160° for 3 hours but the heating and cooling periods were extended to correspond more closely with the work of Pearl (20), the details of which are found in a later section.

In each case, the contents of the cooled bomb were rinsed out with several portions of 5% sodium hydroxide, the last rinse being essentially colorless. The reaction mixtures were dark brown in color and had a

strong odor of nitrobenzene. Droplets of a heavier phase, presumably nitrobenzene and its reduction products, separated from the mixtures.

Table III summarizes the temperature schedules which were followed.

TABLE III

SUMMARY OF OXIDATIONS

	1	2	3	4	5	6	7	8	9
Weight of starting materials, g.	7.5	7.5	22.5[b]	79.0[c]	7.5	7.5	6.8	7.5	7.5
Time to temperature, min.	45	45	45	40	45	45	45	45	45
Reaction temperature	160	160	160	160	160	160	160	180	160
Time at temperature, hrs.	3	3	3	3	3	3	3	2	3
Cooling time to 80°, min.	60	60	60	150	60	60	60	90	120
Yield of vanillin, %[d]	--	--	--	3.7	75	--	--	0.9	0.6
Yield of 6-chloro-vanillin, %	--	--	1	2.3	--	81	--	2.3	2.3
Yield of vanillic acid, %	2	--	--	5.3	0.8	--	74	4.5	4.2

a. Starting materials used were as follows:
   Oxidations 1-3 -- Barton's chlorite lignin A
   Oxidation 4 -- Bublitz' chlorite lignin
   Oxidation 5 -- Vanillin
   Oxidation 6 -- 6-Chlorovanillin
   Oxidation 7 -- Vanillic Acid
   Oxidations 8,9 -- Barton's chlorite lignin A

b. Oxidation 3 was carried out in three separate reactions, each on 7.5 grams of starting material.

c. Oxidation 4 was carried out in a stirring autoclave; all others were run in a rocking bomb.

d. All yields are based on the weight of starting material.

ORIENTATIVE OXIDATIONS

OXIDATION 1

Oxidation 1 was performed on 7.5 grams of Barton's chlorite lignin A; a composite of several smaller samples was used on which no analysis for methoxyl or chlorine was made (see Table III for oxidation conditions). The reaction products were subjected first to steam distillation and then to repeated extraction with benzene for the purpose of removing unreacted nitrobenzene and its reduction products. The extracted solution was acidified with sulfuric acid which caused the formation of a small amount of dark brown solid. Repeated trituration of the gummy solid with ether converted it to a fine powder weighing 0.75 gram.

The acidic solution was extracted seven times with 200-ml. portions of ether. The ether extracts, combined with the ether triturate described above, were concentrated to 250 ml. and extracted successively with four 100-ml. portions of 21% sodium bisulfite solution, four 100-ml. portions of saturated sodium bicarbonate solution and, finally, four 100-ml. portions of 5% sodium hydroxide solution. The neutral components remaining in the ether solution were discarded.

Each fraction was acidified with sulfuric acid and extracted with ether. The ether extracts were dried over sodium sulfate and evaporated to yield residues, the weights of which are summarized as follows:

Fraction	Weight, g.
Ether-insoluble solid	0.75
Bisulfite-soluble fraction	1.25
Bicarbonate-soluble fraction	0.5
Sodium hydroxide-soluble fraction	0.1
Neutral fraction	not weighed
Total	2.6

The only compound isolated from Oxidation 1 was vanillic acid. It was obtained from the bicarbonate-soluble fraction by applying an aliquot of its chloroform-soluble portion to a column of acid-washed Magnesol (21) and developing the chromatogram with 100:1 benzene-ethanol. The major zone, as determined by streaking the extruded column with solutions of 2,4-dinitrophenylhydrazine, ferric chloride, and potassium permanganate, was eluted with acetone and yielded a product melting at 171 to 190°. Two crystallizations from chloroform raised the melting point to 203 to 205°. A mixed melting point with an authentic sample of vanillic acid was not depressed. The yield of vanillic acid based on chlorite lignin was calculated to be about 2%. In addition, a small amount of vanillic acid was recovered from the bisulfite-soluble fraction by chromatographic techniques.

Principally, however, the products of Oxidation 1 served as materials with which to practice various chromatographic techniques; a total of seven chromatograms on acid-washed Magnesol was attempted, using different solvents, developers, and methods of application. It was shown that neither benzene, chloroform, petroleum ether, nor ethyl acetate was capable of yielding crystalline materials directly from the bisulfite-soluble fraction.

OXIDATION 2

The isolation of vanillic acid from both the bisulfite-soluble and bicarbonate-soluble fractions of Oxidation 1 suggested a reversal of the order of these two extractions in the separation of the products of Oxidation 2. The oxidation was performed on 7.5 grams of Barton's chlorite lignin A described in Oxidation 1. The separation procedure followed that outlined in Oxidation 1, with the added variation that the steam distillation was omitted. The following fractions were obtained:

Fraction	Weight, g.
Ether-insoluble solid	0.65
Bicarbonate-soluble fraction	2.62
Bisulfite-soluble fraction	0.02
Sodium hydroxide-soluble fraction	0.30
Neutral fraction	0.03
Total	3.62

The bicarbonate-soluble fraction containing the bulk of the material was further fractionated by trituration with various combinations of benzene, chloroform, and ether, by steam distillation, and by sublimation. No chromatographic separations were attempted. A small amount of vanillic acid was recovered.

The benzene-soluble portion of the phenolic fraction was applied to an acid-washed Magnesol column and developed with 100:1 petroleum ether-ethanol. The material eluted from the major zone was crystallized from petroleum ether and carbon tetrachloride to yield orange crystals melting at 150 to 152°. The compound was identified as p-hydroxyazobenzene, a nitrobenzene reduction product; no depression was observed in a mixed melting point with an authentic sample and the ultraviolet absorption spectrum was

found to coincide with that reported in the literature (22). The formation of p-hydroxyazobenzene from nitrobenzene is not to be attributed to the specific reducing action of chlorite lignin; Freudenberg and coworkers (9) and Leopold (13) have reported its formation in the nitrobenzene oxidation of other lignin preparations or lignin model compounds.

OXIDATION 3

The appreciable water solubility of some of the lignin oxidation products may cause them to appear in fractions other than those which their functional groups would dictate when the separation is based on partition between ethereal and aqueous phases. Accordingly, the separation of the products of Oxidation 3 was undertaken by a method which should depend solely upon differences in acidity. For this purpose, the use of an anion-exchange column was developed (described in a later section).

In order to have a larger quantity of material with which to work, the products of three reactions (obtained by the method of Oxidations 1 and 2) were combined, utilizing a total of 22.5 grams of Barton's chlorite lignin A. The products of the reaction were first subjected to steam distillation and benzene extraction to remove unreacted nitrobenzene and its reduction products. When the mixture was acidified with 1:1 sulfuric acid a gummy brown solid was formed which became powdery after repeated trituration with ether. This ether-insoluble solid had a weight of 4.02 grams.

The acidified solution was saturated with sodium chloride and extracted with ether in an air-agitated continuous extractor (23). The ether extract,

combined with the ether triturate described above, was dried and concentrated, leaving a residue weighing 11.0 grams. This ether-soluble fraction was dissolved in 110 ml. of absolute ethanol, diluted with 110 ml. of water, and passed through an anion-exchange column which was intended to retain the acidic products but to pass the phenolic constituents.

The column consisted of 60 ml. of Duolite A-3 resin contained in a 21.5-mm. diameter glass tube. It had been prepared for the operation by successive treatments with N sodium hydroxide, distilled water, and 50% ethanol. The application of the ether-soluble fraction to the column was followed by 1150 ml. of 50% ethanol.

In order to recover the phenolic materials the effluent was concentrated to remove ethanol, saturated with sodium chloride, and extracted with ether. The ether extracts were combined and successively extracted with 21% sodium bisulfite and 5% sodium hydroxide solutions. The neutral materials remaining in the ether solution weighed 44 mg. and were not further examined.

The combined bisulfite extracts were acidified with 1:1 sulfuric acid, heated on the steam bath, and extracted with ether; the dried ether extracts yielded 0.78 gram of bisulfite-soluble material. However, the residual acidic solution after this treatment deposited a white, ether-soluble precipitate over a period of time, evidently because of the incomplete breakdown of the bisulfite addition complex. Accordingly, the acidic solution was boiled for one hour, cooled, and extracted with ether; an additional quantity of bisulfite-soluble material was recovered. This secondary product was crystallized from water to yield 80 mg. of crystals

melting at 158 to 168°. These crystals were used to seed the hot water-soluble portion of the main bisulfite-soluble fraction, inducing the formation of 158 mg. of crystals melting at 131 to 158°. This product was dissolved in chloroform, applied to a column of acid-washed Magnesol, and developed with 100:1 benzene-ethanol. The main zone, upon elution with acetone, yielded 92 mg. of crystals melting at 150 to 158°. Crystallization from dilute ethanol raised the melting point to 165 to 167.5°. A mixed melting point with an authentic sample of 6-chlorovanillin was not depressed.

The mother liquors from these crystallizations were combined and analyzed by the paper partition chromatographic technique described in a later section. Vanillin and 6-chlorovanillin were shown to be present but there was no evidence for the presence of 5-chlorovanillin.

The phenolic materials, recovered from the 5% sodium hydroxide extracts by acidification and ether extraction, weighed 0.76 gram and were found to be partly volatile with steam. The steam distillate was light yellow in color; extraction with a small amount of ether concentrated the color in the ether layer. A total of 13 liters of distillate was obtained before coming over colorless. The distillate was extracted with ether; the dried ether extract yielded 23 mg. of p-hydroxyazobenzene as orange crystals melting at 153 to 154°. An additional 56 mg. of crude p-hydroxyazobenzene melting at 145 to 149° was recovered from the distillation flask residue by trituration with petroleum ether. This product was purified by several crystallizations from petroleum ether and carbon tetrachloride, raising the

melting point to 153.5 to 154.5°. Efforts to obtain crystalline products from the petroleum ether-insoluble phenolic material by chromatographic separations or by the use of different crystallizing solvents were not successful.

The acidic materials retained on the anion exchange column were removed by elution with 190 ml. of 5% sodium hydroxide solution. Acidification with 1:1 sulfuric acid caused the formation of a dark brown oil which became powdery after repeated trituration with ether and weighed 0.59 gram. The ether-soluble material failed to yield crystalline products despite attempts at fractionation, including triturations with chloroform and benzene, steam distillation, and extraction with saturated sodium bicarbonate solution.

DISCUSSION OF ORIENTATIVE OXIDATIONS

The results of the orientative work may be summarized as follows.

1. Vanillic acid and 6-chlorovanillin were isolated as crystalline products originating from the chlorite lignin. Vanillin was shown to be a third product on the basis of a paper partition chromatogram. p-Hydroxyazobenzene (a nitrobenzene reduction product) was isolated from the phenolic fractions of the ether-soluble products.

2. The failure of the anion-exchange resin technique to yield an acidic fraction from which crystalline compounds, specifically vanillic acid, could be isolated caused this approach to be discarded despite the promise shown in work with pure compounds discussed in a later section. However, it did serve the useful purpose of permitting the first isolation of 6-chlorovanillin in this work.

3. The successive extractions with bisulfite bicarbonate, and sodium hydroxide solutions, in that order, appeared to offer the most promise as a means of separating the products of the reaction.

4. The yields of ether-soluble oxidation products appeared to be of the same degree of magnitude as those reported from alkaline nitrobenzene oxidations of other lignin preparations. These yields, uncorrected for p-hydroxyazobenzene and based on chlorite lignin, were 25, 40, and 49% in Oxidations 1, 2, and 3, respectively. Similarly, yields of 11 and 18% of an ether-insoluble acidic material were observed. This substance, high-melting and containing halogen, was probably a complex product stemming either from a resynthesis of simple oxidation products or from an incomplete fragmentation or modification of the chlorite lignin itself.

OXIDATION 4 (LARGE-SCALE OXIDATION)

On the basis of the information obtained in the orientative oxidations, plans were made to undertake a large-scale oxidation. A total of 79 grams of chlorite lignin prepared by Bublitz (4) from black spruce was used. A detailed description of the preparation of this product and the oxidation procedure is found in an earlier section.

The major separation of the oxidation products into bisulfite-, bicarbonate-, and sodium hydroxide-soluble fractions was accomplished in the manner previously discussed. Refinements in the separation techniques included the use of powdered cellulose column chromatography for the separation of vanillin and 6-chlorovanillin from one another and for the more quantitative isolation of vanillic acid from the bicarbonate-soluble fraction.

Isolation of Major Fractions

The reaction mixture, in two equal portions, was steam distilled for 4 hours, saturated with sodium chloride, and extracted seven times with ether in a separatory funnel for the purpose of removing nitrobenzene and its reduction products.

Acidification was then accomplished in an ice bath with 33% sulfuric acid at 0°. A gummy material which appeared was triturated repeatedly with ether until it became a reddish-brown powder. The ether solution was added to the ether extract obtained from a 24-hour treatment of the acidified reaction mixture in an air-agitated continuous extractor.

The acid- and ether-insoluble powder, weighing 16.4 grams, had a methoxyl content of 3.5% and a chlorine content of 21.6%. A portion of the crude product was freed of inorganic material by triturating the dry powder with anhydrous dioxane and precipitating the filtered triturate into anhydrous ether. The purified product had a methoxyl content of 9.8% and a chlorine content of 3.5%.

A 25-ml. aliquot of the ether-soluble products was taken from the combined and concentrated ether extracts (440 ml.). It was concentrated to dryness and analyzed for methoxyl and chlorine content. Calculations showed the total ether soluble fraction to have a weight of 38.9 grams and to contain 13.0% of methoxyl and 2.9% of chlorine.

The 415 ml. of ether solution remaining after removal of the aliquot were extracted successively with ten 150-ml. portions of 21% sodium bisulfite

solution, ten 150-ml. portions of saturated sodium bicarbonate solution, and eight 150-ml. portions of sodium hydroxide solution. The combined extracts in each case were acidified with 33% sulfuric acid and, in the case of the bisulfite-soluble fraction, boiled until sulfur dioxide removal was complete. The acidified fractions were then extracted with ether; the ether extracts were washed with water and dried over sodium sulfate.

Upon acidification, the bisulfite-soluble fraction yielded a material which, despite repeated trituration with ether, could not be redissolved. It had a weight of 3.7 grams and a methoxyl content of 3.5%. It is probable that materials of this type make up the difference between the total yield of ether-soluble materials predicted on the basis of the aliquot (38.9 grams) and the sum of the weights of the materials actually recovered after undergoing solution in a basic medium, acidification, and re-extraction with ether (30.2 grams).

Bisulfite-soluble Fraction of Oxidation 4

Evaporation of the ether extracts of the acidified sodium bisulfite solution yielded 16.46 grams of a brown powder. This was triturated with benzene and a small amount of insoluble material removed by filtration. A part of the residue obtained by evaporation of the benzene solution was then fractionated by application to a column of acid-washed Magnesol. The contents of similar zones from two chromatograms were combined. The material was applied to the column in a benzene solution and developed with 100:1 petroleum ether-ethanol. The pertinent data on these separations are listed below.

	Run 1	Run 2
Weight applied, mg.	132	929
Column diameter, mm.	20	35
Column length, cm.	17	19
Developer volume, ml.	100	750

After streaking the extruded columns with potassium permanganate, ferric chloride, and 2,4-dinitrophenylhydrazine solutions, they were divided into zones, each of which was eluted with acetone. The results of these separations are given below.

Zone	Recovered Material, mg.	%
A	312	29.4
B	209	19.7
C	439	41.3
Total	960	90.4

Each fraction, which upon slow evaporation of its ethanolic solution yielded crystalline material, was spotted on a paper chromatogram and developed with butanol-aqueous ammonia*. Fraction C was shown to be predominantly vanillin and 6-chlorovanillin; Fraction A was chiefly vanillic acid; Fraction B showed the presence of all three compounds.

Fraction A. The material in Fraction A was first triturated with petroleum ether and the residue in turn was triturated with benzene, leaving an insoluble residue weighing 65 mg. The benzene- and petroleum ether-soluble materials were combined (weight 223 mg.) and applied in benzene to a column of acid-washed Magnesol (20 mm. in diameter and 170 mm. in length).

* The butanol-aqueous ammonia referred to throughout this work was prepared by saturating normal butanol with an aqueous solution containing approximately 2% ammonia as NH_3. It was obtained by taking the upper layer of a thoroughly agitated mixture of 1000 ml. of butanol, 560 ml. of distilled water, and 44 ml. of concentrated ammonium hydroxide.

Development was accomplished with 75 ml. of 20:1 petroleum ether-ethanol. Streaking reagents indicated separation into four zones which, upon elution with acetone, yielded the following fractions:

Zone	Recovered Material, mg.	%
Aa	54	24.2
Ab	98	44.0
Ac	35	15.7
Ad	18	8.1
Total	205	92.0

Fraction Ac, melting at 185 to 195°, was sublimed at 100 to 120°/0.4 mm. to yield a sublimate which melted at 195 to 199° and showed no depression of melting point when mixed with an authentic sample of vanillic acid.

Ad had a strong odor of vanillin but was not analyzed.

Aa and Ab were combined; trituration with benzene dissolved about half of the material. Application of this benzene solution to a column of acid-washed Magnesol yielded zones corresponding to vanillin and vanillic acid which were not further investigated.

Fraction B. The material in Fraction B was crystallized from chloroform and yielded 52 mg. of tan crystals melting at 190 to 199°. Two further crystallizations from water raised the melting point to 197.5 to 200°; a mixed melting point with an authentic sample of vanillic acid showed no depression.

The material recovered from the combined mother liquors of these crystallizations, weighing 107 mg., was chromatographed on acid-washed

Magnesol and developed with 100:1 petroleum ether-ethanol. The main zone contained 76 mg. of material; after crystallization from chloroform, it had a melting point of 194 to 200° and was presumed to be crude vanillic acid from its chromatographic behavior.

Fraction C. The two chief components of this fraction, vanillin and 6-chlorovanillin, were separated on a column of powdered cellulose. Whatman Standard Grade powdered cellulose was packed in ten sections in a glass tube. The column itself was 30 mm. in diameter and 360 mm. in length. Each layer was thoroughly tamped into place with a cork plunger. A perforated porcelain plate covered with glass wool supported the bottom of the column.

The column was washed successively with 500 ml. of water, 500 ml. of 50% ethanol, and 500 ml. of butanol-aqueous ammonia, the top of the column never being allowed to run dry. The developer had a flow rate of approximately 50 ml. per hour.

A portion of Fraction C weighing 300 mg. was intimately mixed with 800 mg. of powdered cellulose and applied in a uniform layer to the top of the column. Butanol-aqueous ammonia developer was then applied. The eluate was checked periodically by collecting a drop on a filter paper and treating it with 2,4-dinitrophenylhydrazine reagent. Vanillin and 6-chlorovanillin gave colors differing slightly in shade. The first carbonyl test was obtained at 3 hours. The fractions obtained by evaporation of the eluate under reduced pressure are shown below.

Zone	Time of Collection, hrs.	Weight mg.	Melting Point, °C.
Ca	3.0-3.3	16	152-162
Cb	3.3-4.0	98	74-78
Cc	4.0-4.5	160	
Cd	4.5-5.0	12	
Ce	5.0-5.5	5	
Cf	5.5-7.0	-	
Cg	7.0-15.0	11	
Ch	15.0-24.0	11	

Cb was triturated with petroleum ether; 19 mg. of insoluble material were removed by filtration. The filtrate yielded 46 mg. of tan crystals melting at 163.5 to 168.5°. A mixed melting point with an authentic sample of 6-chlorovanillin was not depressed.

Cc was triturated with petroleum ether; 32 mg. of insoluble material were removed by filtration. The filtrate yielded 92 mg. of white crystals melting at 76 to 76.5°. A mixed melting point with an authentic sample of vanillin showed no depression.

The yields of isolated crystalline compounds, based on chlorite lignin, were calculated as follows:

Vanillin (Cc)

$$\frac{(160 - 32)}{(300)} \times \frac{(439)}{(132 + 929)} \times \frac{(16.46)(1000)}{(79.0)(1000)} \times 100 = 3.7\%$$

6-Chlorovanillin (Cb)

$$\frac{(98 - 19)}{(300)} \times \frac{(439)}{(132 + 929)} \times \frac{(16.46)(1000)}{(79.0)\ (1000)} \times 100 = 2.3\%$$

Vanillic Acid (Ac and B)

$$\frac{(35 + 52 + 76)}{(132 + 929)} \times \frac{(16.46)(1000)}{(79.0)\ (1000)} \times 100 = 3.2\%$$

Acid Fraction

Concentration of the ether extracts of the acidified sodium bicarbonate solution yielded 5.6 grams of light brown powder. Crystals could not be obtained from benzene or ethanol solutions but concentration of a chloroform solution gave 0.8 gram of crude vanillic acid melting at 185 to 190° (Fraction A). The remaining 4.8 grams of chloroform-soluble material comprised Fraction B.

Fraction A was subjected to successive sublimations at 0.2 mm. and yielded the following fractions:

Fraction	Sublimation Time, hrs.	Sublimation Temp., °C.	Sublimate g.	Sublimate m.p., °C.
Aa	42	98	0.1	190-197
Ab	3	135	0.05	194-200
Ac	6	120	0.1	195-204
Ad	15	120	0.15	195-204
Ae	17	130	0.25	200-205
Af	7	145	0.05	195-201
residue			0.15	170-215

Ab was crystallized from water to yield a product melting at 203 to 205°.

Ac and Ad were combined and crystallized from chloroform to yield 125 mg. of crystals melting at 204.5 to 206°; a mixed melting point with an authentic sample of vanillic acid was not depressed.

A portion of Fraction B weighing 754 mg. was then subjected to a similar sublimation at 0.2 mm. to give the following sublimates:

Fraction	Sublimation Time, hrs.	Sublimation Temp., °C.	Sublimate, mg.	m.p., °C.
Ba	2	115-120	77	150-180
Bb	3	120	55	185-192
Bc	3	120	35	184-193
residue			608	

Ba, Bb, and Bc were recombined and an attempt was made to obtain a chromatographic separation by application in a benzene solution to a column of acid-washed Magnesol. Development with 100:1 petroleum ether-ethanol concentrated 71% of the material into a zone which, when eluted, gave a product melting at 195 to 200°.

At this point, the impracticality of separating vanillic acid and 6-chlorovanillic acid by sublimation was demonstrated by subliming at 110°/0.2 mm. a mixture of two compounds (3:2 parts by weight) melting at 185 to 190°. The sublimate melted at 185 to 193°, showing no appreciable separation had occurred.

Ba was spotted on a paper chromatogram and developed with butanol-aqueous ammonia. Four visible spots at R_F 0.24, 0.54, 0.62, and 0.96 were found, as well as additional spots visible under ultraviolet light.

Accordingly, a column of powdered cellulose was prepared in a tapered glass tube by the method previously described; the column had a diameter of 35 mm. and a length of 200 mm. The column was completely prewet with the butanol-aqueous ammonia developer at which time 442 mg. of B, dissolved in 25 ml. of warm developer, were applied to the column. After 4 hours' development, the lowest visible colored zone had reached the bottom of the

column and the application of developer was halted. When the column had drained it was extruded and cut into sections under ultraviolet light. The following nine fractions were obtained by elution of the zones with acetone:

Fraction	Recovered Material, mg.	m.p., °C.
Bd	2	
Be	3	
Bf	6	
Bg	50	190-197
Bh	30	200-204
Bi	9	
Bj	13	
Bk	11	
effluent	130	
	254	

The low recovery of material applied (57%) is believed to be due to the inability of the eluting agent, acetone, to overcome the adsorptive powers of the cellulose. The eluted powder was brown in color despite continued elution with acetone. Later work showed that 50% ethanol would have been a better choice of eluting agent.

A similar isolation of a fraction rich in vanillic acid was made by substituting a 180-mm. strip of heavy filter paper (Whatman 3M) for the powdered cellulose column. A heavy syrup of B in warm butanol was repeatedly applied from a fine capillary to a narrow zone near the top of the strip; the chromatogram was developed with butanol-aqueous ammonia for 10 hours, during which time the solvent front moved 321 mm. The developed sheet was examined under visible and ultraviolet light and a narrow strip cut down the center was sprayed with bis-diazotized benzidine.

A number of well-defined bands were found and the paper was cut accordingly. The strips so obtained were eluted with 50% ethanol by the Dent technique (24), yielding the following distribution of fractions:

Fraction	mg.	R_f	Colors Shown, visible	ultraviolet	spray
Ba'	3	0-0.015			
Bb'	2	0.015-0.025			
Bc'	2	0.025-0.06			brown
Bd'	2	0.06-0.08			
Be'	13	0.08-0.22			dark brown
Bf'	3	0.22-0.24	brown		
Bg'	2	0.24-0.27		intense blue	brown-orange
Bh'	0	0.27-0.28	yellow		
Bi'	2	0.28-0.34			
Bj'	3	0.34-0.44			
Bk'	3	0.44-0.55			
Bl'	2	0.55-0.64	yellow		brown
Bm'	3	0.64-0.70			
Bn'	4	0.70-0.86			
Bo'	1	0.86-0.94	yellow		brown
Bp'	3	0.94-1.0			

Be', presumed to be predominantly vanillic acid, had a melting point of 180 to 190°.

The estimated yield of vanillic acid obtained from the acid fraction and based on chlorite lignin was calculated as follows:

Vanillic acid (a, Bg, and Bh)

$$\left\{\frac{(0.8)}{(5.6)} + \left[\frac{(6.030 + 0.050)}{(0.442)} \times \frac{(5.6 - 0.8)}{(5.6)}\right]\right\} \times \frac{(5.6)}{(79.0)} \times 100 = 2.1\%$$

As in the isolation of vanillic acid from the bisulfite-soluble fraction (3.2% based on chlorite lignin), a positive Beilstein test for halogen in the crude product suggested that 6-chlorovanillic acid may have accompanied vanillic acid in all the separations attempted.

Phenol Fraction of Oxidation 4

Concentration of the ether extracts of the acidified sodium hydroxide solution yielded 3.7 grams of a viscous brown oil. Trituration with petroleum ether dissolved a portion of the fraction and successive concentration and cooling yielded crystalline materials melting at 146 to 151°, 144 to 149°, and 139 to 148°. These crystalline fractions, weighing 2.6 grams, were combined. A single crystallization from glacial acetic acid gave 1.15 grams of orange crystals melting at 150 to 151.5°. They exhibited a negative Beilstein test for halogen and did not depress a mixed melting point with an authentic sample of p-hydroxyazobenzene.

The contents of the mother liquors from the above crystallizations were applied to an acid-washed Magnesol column and developed with petroleum ether, producing a very complex chromatogram. One zone, when eluted, yielded 0.1 gram of an oil smelling strongly of guaiacol. Efforts to produce a crystalline benzoate by the Schotten-Baumann reaction were not successful.

Discussion of Oxidation 4

The recovery of ether-soluble oxidation products from Oxidation 4 was of the same order of magnitude as that found in previous oxidations. On the basis of the aliquot taken from the total ether-soluble fraction, 38.9 grams of ether-soluble products were obtained originally although the sum of the subsequently fractionated products, as shown below, actually totalled 30.2 grams.

Fraction	Weight, g.
Bisulfite-soluble fraction	
Ether-soluble	16.5
Ether-insoluble	3.7
Bicarbonate-soluble fraction	5.6
Sodium hydroxide-soluble fraction	3.7
Neutral fraction	0.7
Total	30.2

If the weight of ether-soluble oxidation products (38.9 grams) is corrected for the yield of p-hydroxyazobenzene (2.6 grams) which cannot be attributed to the chlorite lignin, the yield of ether-soluble oxidation products to be attributed to chlorite lignin and based on chlorite lignin is 46%.

The methoxyl content of the total ether-soluble fraction (13.0%) was slightly higher than that of the original chlorite lignin (12.5%). When the methoxyl content of the acidic but ether-insoluble fraction is also taken into consideration (3.5% of 16.4 grams), it is seen that the recovery of methoxyl groups in the nonvolatile acidic products of the oxidation, based on chlorite lignin, was

$$\frac{(0.035)\ (16.4) + (0.13)\ (38.9)}{(79.0)\ (0.125)} \times (100) = 57\%.$$

Oxidations of Vanillin, 6-Chlorovanillin, and Vanillic Acid

Oxidations 5, 6, and 7 comprised a brief study of the effect of alkaline nitrobenzene oxidation conditions on the major products of the chlorite lignin oxidation--i.e., vanillin, 6-chlorovanillin, and vanillic acid. Each of these compounds was subjected to conditions approximating those used in Oxidations 1 through 4 (see Table III). In this way answers were obtained to two important questions.

1. How resistant are these compounds to further oxidation and/or degradation once they have been formed from a lignin oxidation? The actual yields may be significantly less than the theoretical values to be obtained if the compounds do not undergo further reaction.

2. To what extent does vanillic acid (or 6-chlorovanillic acid) result from the further oxidation of vanillin (or 6-chlorovanillin)?

The products of each oxidation were subjected to steam distillation for 2 hours and extracted five times with ether (totalling 2 liters in volume) to remove nitrobenzene and its reduction products.

Products of Vanillin Oxidation (Oxidation 5)

The aqueous alkaline solution, freed of nitrobenzene and its reduction products, was acidified with 1:1 sulfuric acid and extracted with ether for 10 hours in an air-agitated continuous extractor. The dark brown ether extract was filtered, dried with sodium sulfate, and the ether was removed by distillation, leaving 8.75 grams of dark reddish-brown oily solid having an odor of guaiacol.

This product was dissolved in 150 ml. of 10% sodium hydroxide and the solution was saturated with sulfur dioxide. After extraction with ether, the bisulfite-soluble materials were liberated from the solution by acidification with 1:1 sulfuric acid, followed by aeration and heating on the steam bath. Cooling produced an oil which soon solidified; a total of 6.6 grams of crude vanillin melting at 68 to 76° was recovered by repeated ether extraction of the sodium chloride-saturated acidified solution.

A portion of the crude vanillin weighing 1.63 grams was thoroughly triturated with petroleum ether; a dark brown residue weighing 0.17 gram could not be dissolved. The soluble portion yielded crystals of vanillin weighing 1.31 grams and melting at 78 to 80°; a second crop of crystals weighed 0.09 gram and melted at 76 to 78°.

Thus, the minimum yield of unreacted vanillin was calculated to be

$$\frac{(1.31 + 0.09)}{(1.63)} \times \frac{(6.6)}{(7.5)} \times (100) = 75\%.$$

Another portion of the crude vanillin product weighing 4.70 grams was triturated repeatedly with hot benzene; a residue of 0.10 gram remained. The benzene-soluble material (4.60 grams) was applied to a column of acid-washed Magnesol 45 mm. in diameter and 240 mm. in length. Development was accomplished with 600 ml. of 100:1 benzene-ethanol. The fractions which were obtained by acetone elution of the various zones are listed below:

Zone	Recovered Material,	
	g.	%
A	3.60	78.4
B	0.11	2.4
C	0.19	4.1
Total	3.90	84.9

Fraction A was found to be vanillin melting at 78.5 to 80.5°.

Zone B exhibited a strong green color when the column was streaked with ferric chloride solution, suggesting the presence of an ortho-dihydroxy compound such as demethylated vanillin--i.e., protocatechualdehyde. Accordingly, Fraction B was spotted on paper, developed with butanol-aqueous

ammonia, and sprayed with bis-diazotized benzidine. In addition to spots corresponding to vanillin and vanillic acid, the chromatogram showed a spot at R_F 0.32 which exhibited a light tan color when sprayed; its behavior was identical in every way to that shown by an authentic sample of protocatechualdehyde. Two unidentified spots were also found at R_F 0.00 and 0.03.

The ether extract of the sulfur dioxide-saturated alkaline solution of the oxidation products was dried and concentrated to yield 1.1 grams of material. A paper chromatogram developed with butanol-aqueous ammonia yielded spots corresponding to vanillin and vanillic acid. A benzene solution of this fraction yielded 60 mg. of crude vanillic acid melting at 180 to 195°. Recrystallization raised the melting point to 200 to 203°.

Products of 6-Chlorovanillin Oxidation (Oxidation 6)

The reaction mixture obtained from the 6-chlorovanillin oxidation contained a large amount of solid material (Fraction A), which was unchanged during the steam distillation. It was separated from the distillation flask residue by filtration and was washed thoroughly with 2 N sodium hydroxide and finally with water. Acidification of this water washing with 1:1 sulfuric acid yielded 0.8 gram of 6-chlorovanillin melting at 165 to 168°. Fraction A [weighing in excess of 5 grams (Fraction B)] was high melting and left an alkaline residue upon ignition; its behavior suggested it to be the sodium salt of 6-chlorovanillin.

Accordingly, the salt was dissolved in hot dilute ethanol and acidified with 1:1 sulfuric acid. The cooled solution yielded 4.35 grams of

6-chlorovanillin melting at 162 to 167° (Fraction C). Concentration and cooling of the mother liquor yielded a second crop of crystals melting at 160 to 168° and weighing 0.30 gram (Fraction D).

The three crude 6-chlorovanillin fractions (B, C, and D) (totalling 5.45 grams) were combined. A portion weighing 891 mg. was dissolved in ethanol and decolorized with charcoal. Crystals were obtained from dilute ethanol melting at 167 to 168° and weighing 820 mg., showing the crude product to be at least 92% pure.

The alkaline filtrate obtained after separation of the sodium salt was extracted five times with ether (totalling 2 liters in volume) to remove nitrobenzene reduction products. Acidification of the aqueous alkaline solution with 1:1 sulfuric acid was followed by 10 hours' extraction with ether in an air-agitated continuous extractor.

One half of the extracted aqueous phase was further acidified with nitric acid and an excess of silver nitrate solution was added. The resulting silver chloride precipitate was allowed to stand overnight and was then collected on a tared fritted glass crucible. The precipitate was washed with hot dilute nitric acid and water and dried to constant weight in a 110° oven; its weight was 0.144 gram. Calculations showed that approximately 5% of the chlorine present in the 6-chlorovanillin was converted to chloride ion during the oxidation.

The ether extract obtained from the continuous extractor was in turn extracted with 21% sodium bisulfite solution and finally washed with water,

dried with sodium sulfate, and the ether distilled to yield 0.43 gram of material. A paper chromatogram of this fraction developed with butanol-aqueous ammonia showed spots corresponding to 6-chlorovanillic acid and 6-chlorovanillin, as well as a number of other unidentified spots.

The bisulfite extract was acidified with 1:1 sulfuric acid and aerated while being heated on the steam bath. A light brown precipitate of 6-chlorovanillin formed which weighed 965 mg. and melted from 165 to 167.5°. The filtrate remaining after collection of this fraction was extracted with ether; the dried extract, after distillation of the ether, weighed 0.24 gram. When applied to a paper chromatogram and developed with butanol-aqueous ammonia, this residue exhibited spots corresponding to 6-chloro-vanillin and 6-chlorovanillic acid.

This residue was accordingly triturated with hot benzene, leaving an insoluble dark brown tarry residue weighing 0.11 gram. The soluble portion (weighing 140 mg.) was applied to a column of acid-washed Magnesol 20 mm. in diameter and 176 mm. in length. The chromatogram was developed with 125 ml. of 100:1 benzene-ethanol. The five fractions obtained by acetone elution are shown below:

Zone	Recovered Material, mg.	%
A	18	12.9
B	7	5.0
C	40	28.6
D	12	8.6
E and effluent	46	32.8
	123	87.9

Fractions A, B, and D were discarded. Fraction E was shown to be 6-chlorovanillin melting at 165 to 166.5°.

Zone C, when streaked with ferric chloride, exhibited the same strong green color shown by the product of the vanillin oxidation and identified as protocatechualdehyde. Accordingly, the chromatographic behavior of Fraction C was compared with that of 6-chloroprotocatechualdehyde prepared by the demethylation of 6-chlorovanillin. Their behavior was identical in every respect--i.e., butanol-aqueous ammonia moved the spots the same distance (R_F of 0.47 in a 3.5-hour run) and a ferric chloride spray produced the same green color. In addition, the presence of 6-chlorovanillic acid was indicated in Fraction C. Fraction C had a melting point of 167 to 175° as recovered from the column. A single crystallization from chloroform raised the melting point to 187 to 195°.

The minimum yield of 6-chlorovanillin recovered unchanged from the oxidation was calculated to be

$$\frac{(5.45)\ (0.92) + (0.965) + (0.046)}{(7.50)} \times (100) = 81\%.$$

Synthesis of 6-Chloroprotocatechualdehyde

Protocatechualdehyde has been prepared by the demethylation of vanillin with anhydrous aluminum bromide in 93% yield (25). A similar procedure was used in this work to obtain the corresponding demethylated product from 6-chlorovanillin.

To a solution of 9.3 grams (0.05 mole) of 6-chlorovanillin dissolved in 100 ml. of nitrobenzene at 95° was added at once a solution of 26.7 grams (0.1 mole) of anhydrous aluminum bromide in 50 ml. of nitrobenzene. A temperature of 85 to 95° was maintained for one hour with frequent stirring. The mixture was cooled and poured into 500 ml. of water containing a little hydrochloric acid. The mixture was extracted with ether and the ether, in turn, was extracted with 5% sodium hydroxide. The sodium hydroxide extract was washed with ether and acidified with 1:1 sulfuric acid. The acidified solution was extracted with ether; after drying with sodium sulfate, the ether was distilled, yielding a reddish-brown solid melting at 180 to 195°. This product was dissolved in ethanol, decolorized with charcoal, and diluted with benzene. A light reddish-brown solid was obtained melting at 208 to 211°. Crystallization of a portion from benzene raised the melting point to 212 to 213°. Weisse reported a melting point of 211° for 6-chloroprotocatechualdehyde prepared from 6-chloropiperonal (26). The compound was readily crystallized from water. An intense green coloration was obtained when an aqueous or alcoholic solution of the compound was treated with a ferric chloride solution. A strong test for halogen was obtained by the Beilstein test.

Analysis. Calculated for 6-chloroprotocatechualdehyde, $C_7H_5O_3Cl$: C, 48.72; H, 2.92. Found: C, 48.60; H, 3.00.

PRODUCTS OF VANILLIC ACID OXIDATION (OXIDATION 7)

The reaction mixture from the vanillic acid oxidation, after steam distillation and ether extraction, was acidified with 1:1 sulfuric acid.

Crystalline vanillic acid appeared and was collected by filtration; the product weighed 4.50 grams and melted from 204.5 to 206°. The filtrate was extracted with ether and the ether dried and distilled; a residue weighing 1.14 grams remained.

The residue was examined by paper chromatography in which butanol-aqueous ammonia was used as developer. Ferric chloride solution was used as spraying reagent. Vanillic acid appeared to be the chief component of the mixture with an unidentified spot at R_F 0.00 and a visible yellow spot which corresponded to p-hydroxyazobenzene. There was no trace of possible demethylated products, such as protocatechuic acid or pyrocatechol, which were run on the same sheet for comparison purposes. Neither was there any chromatographic evidence of the possible decarboxylated product, guaiacol.

The residue was triturated with benzene; after an insoluble residue was removed by filtration, crystalline vanillic acid weighing 0.50 gram and melting from 205 to 207.5° was obtained. Further trituration of the benzene-insoluble residue with chloroform yielded an additional 0.03 gram of vanillic acid melting at 202 to 205°.

Thus the yield of unreacted vanillic acid recovered from the oxidation was calculated to be

$$\frac{(4.50) + (0.50) + (0.03)}{(6.8)} \times (100) = 74\%.$$

MODIFIED OXIDATION CONDITIONS (OXIDATIONS 8 AND 9)

Oxidations 8 and 9, each using 7.5 grams of Barton's chlorite lignin A (see Table II for analysis), were performed with slightly modified oxidation conditions to reveal the possible influence of small changes in time and temperature on the yields of vanillin, 6-chlorovanillin, and vanillic acid. The separation techniques used were essentially those applied in the large scale Oxidation 4; however, the use of a butanol-aqueous ammonia developer with a powdered cellulose column in the chromatographic separation of the acid fractions revealed a new nitrobenzene reduction product, p-phenylazobenzoic acid.

Oxidation 8 was performed under conditions approximating those recently recommended by Leopold (13), who found that 2 hours at 180° constituted the optimum conditions for the production of vanillin by the alkaline nitrobenzene oxidation of the model compounds with which he worked.

Oxidation 9 retained Freudenberg's original conditions of 3 hours at 160°; however, the heating and cooling periods attendant to the oxidation were extended to more nearly approximate those used in the previously mentioned experiment by Pearl (20). A summary of these conditions is found in Table III.

The major separations of Oxidations 8 and 9 were identical and were achieved as follows.

The usual steam distillation and ether extraction procedure was performed on the products of the reaction to remove nitrobenzene and its

alkaline reduction products. After acidification with cold 1:1 sulfuric acid, the mixture was extracted with ether for 20 hours in an air-agitated continuous extractor. The ether-soluble products were fractionated by successive extractions with five portions of 21% sodium bisulfite solution (totalling 800 ml.), four portions of saturated sodium bicarbonate solution (totalling 800 ml.), and four portions of 5% sodium hydroxide solution (totalling 600 ml.). The combined extracts of each fraction were washed with a small portion of ether which was added to the remaining ether-soluble material prior to the next group of extractions. The residual ether solution was dried with sodium sulfate and evaporated to yield the neutral fraction.

The combined bisulfite extracts were acidified with 1:1 sulfuric acid and heated on the steam bath with aeration until free of sulfur dioxide. The mixture was then saturated with sodium chloride and extracted with ether. The combined ether extracts, after drying with sodium sulfate, were evaporated to yield the bisulfite-soluble fraction.

The bicarbonate- and sodium hydroxide-soluble fractions were obtained in a similar way, with the exception that the sulfur dioxide removal step was omitted.

A comparison of the yields of the major fractions obtained from Oxidations 4, 8, and 9 follows.

Fraction	Yield, % (based on chlorite lignin) 4	8	9
Bisulfite-soluble	25.6	28.4	31.0
Bicarbonate-soluble	7.1	12.0	11.2
Sodium hydroxide-soluble	4.7	5.2	6.1
Neutral	0.9	1.0	4.0
Total	38.3	46.6	52.3

When the relatively large experimental error is considered, it is seen that slight modifications in oxidation conditions did not cause any great changes in the yields of the major fractions; it will also be shown that the yields of crystalline products were essentially unchanged by these modifications (see Table III).

Bisulfite-Soluble Fraction of Oxidation 8

The bisulfite-soluble material from Oxidation 8 (weighing 2.13 grams) was triturated repeatedly with hot chloroform. A residue weighing 0.63 gram could not be dissolved. The combined chloroform triturates (containing 1.50 grams of material) were applied to a column of acid-washed Magnesol 38 mm. in diameter and 200 mm. in length. Development was accomplished with 750 ml. of 100:1 petroleum ether-ethanol. The following fractions were obtained:

Zone	Recovered Material, mg.	%
A	640	42.5
B	215	14.3
C	570	37.9
Total	1425	94.7

Attempts to obtain crystalline products from Fractions A and B were unsuccessful, although paper chromatograms indicated that Fraction B was chiefly vanillic acid with a smaller amount of 6-chlorovanillin.

Fraction C was finely ground and mixed with an equal volume of powdered cellulose. The mixture was applied in a uniform layer to the top of a powdered cellulose column prepared and conditioned in the manner described in Oxidation 4. The flow of the butanol-aqueous ammonia developer was regulated by means of a stopcock at the bottom of the column to 20 to 25 ml. an hour. The liquid flowing from the column was collected in test tubes contained on a circular holder which was automatically advanced at half-hour intervals. In this way, during a 49-hour run, 98 different fractions were obtained. Residual material retained on the column, was removed by elution with 500 ml. of 50% ethanol.

The contents of the first 14 tubes contained only forerun and were discarded. The use of the 2,4-dinitrophenylhydrazine spot test previously described showed that tube 20 represented the change-over point from 6-chlorovanillin to vanillin and that no appreciable amount of vanillin appeared in tubes following tubes following tube 29. This was confirmed by paper chromatograms using butanol-aqueous ammonia as developer. Every fifth tube from 30 to 95 was evaporated to dryness on the steam bath. Only tubes 85, 90, and 95 showed an appreciable residue; paper chromatograms showed the material in these tubes to be chiefly vanillic acid. Consequently, the contents of the following tubes were combined.

Tubes	Recovered Material, mg.	%
15-19	289	50.7
20	12	2.1
21-29	93	16.3
30-80	52	9.1
81-98	13	2.3
50% alcohol eluate	92	16.1
Total	551	96.6

The contents of tubes 15-19, a dark brown residue melting at 145 to 155°, were triturated repeatedly with hot petroleum ether. A residue of 114 mg. was removed by filtering and the filtrate was concentrated and cooled to yield 100 mg. of 6-chlorovanillin as light tan crystals melting at 161 to 166°.

The yield of 6-chlorovanillin based on chlorite lignin was calculated to be

$$\frac{(289 - 114)}{(7.5)(1000)} \times (100) = 2.3\%.$$

The contents of tube 20, a mixture of 6-chlorovanillin and vanillin, were discarded.

The contents of tubes 21-29, a dark brown residue melting at 67 to 75°, were triturated repeatedly with hot petroleum ether, leaving 28 mg. of an insoluble residue. The triturate was concentrated and cooled to yield 34 mg. of crystalline vanillin melting at 74 to 78°.

The yield of vanillin based on chlorite lignin was calculated to be

$$\frac{(93 - 28)}{(7.5)(1000)} \times (100) = 0.9\%.$$

Acid Fraction of Oxidation 8

The bicarbonate-soluble fraction from Oxidation 8 (weighing 0.904 gram) was dissolved in 25 ml. of hot butanol-aqueous ammonia and applied to the powdered cellulose column. Butanol-aqueous ammonia was used as a developer for a period of 65 hours, during which time 130 fractions were automatically collected. The adsorbed material was removed from the column at the end of the run by elution with 500 ml. of 50% ethanol. The first 15 tubes contained forerun and their contents were discarded. Tube 20 and every tenth tube thereafter were evaporated to dryness and spotted on a paper chromatogram which was developed with butanol-aqueous ammonia and sprayed with bis-diazotized benzidine. Vanillic acid was shown to be in tubes 60 to 100. The contents of tube 20 yielded orange crystals upon evaporation. The following fractions were obtained from the run.

Tubes	Recovered Material, mg.	%
16-22	184	20.2
23-53	212	23.5
54-59	105	11.6
60-100	250	27.6
101-130	37	4.2
50% ethanol eluate	195	21.6
Total	983	108.7

The contents of tubes 16-22, an orange crystalline solid, melted in the vicinity of 235°. The material was dissolved in 95% ethanol, treated with charcoal and filtered; the concentrated and cooled filtrate yielded 55 mg. of orange crystals melting at 239 to 241°. Recrystallization did not raise the melting point.

The compound was shown to contain nitrogen by sodium fusion; a Beilstein test for halogen was negative. It dissolved readily in 5% sodium hydroxide to form an orange solution from which it could be precipitated by the addition of dilute sulfuric acid but not by carbon dioxide. No coloration was observed when a ferric chloride solution was added to an ethanolic solution of the compound. When the analysis for carbon and hydrogen was considered in the light of this behavior, it seemed likely that the compound was p-phenylazobenzoic acid; this indentification was confirmed when no depression was observed in a mixed melting point with an authentic sample prepared according to Anspon (27) by the reaction of nitrosobenzene with p-aminobenzoic acid. The ultraviolet absorption spectra of the suspected and authentic samples were shown to coincide.

Analysis. Calculated for p-phenylazobenzoic acid, $C_{13}H_{10}O_2N_2$: C, 69.01; H, 4.45. Found: C, 68.83; H, 4.52.

No crystalline product could be obtained from the contents of tubes 23-53.

The contents of tubes 54-59, melting at 192 to 199°, were repeatedly triturated with hot benzene; a 10 mg. residue remained. Evaporation of the benzene triturate yielded a solid which had a melting point of 200 to 203° and which exhibited a faint Beilstein test for halogen. A mixed melting point with 6-chlorovanillic acid was depressed by 20°, whereas a mixed melting point with vanillic acid showed no depression.

The contents of tubes 60-100 were likewise triturated with hot benzene, which dissolved all except 10 mg. The benzene-soluble portion, melting at

192 to 197°, also exhibited a faint test for halogen but a comparison of mixed melting points showed it to be crude vanillic acid and not 6-chloro-vanillic acid.

The yield of vanillic acid based on chlorite lignin was calculated to be

$$\frac{(250 - 10) + (105 - 10)}{(7.5)\ (1000)} \times (100) = 4.5\%.$$

Phenol Fraction of Oxidation 8

The sodium hydroxide-soluble material (weighing 389 mg.) was triturated repeatedly with hot petroleum ether. A powdery brown residue weighing 155 mg. was removed by filtering. The concentrated and cooled filtrate yielded 159 mg. of crude p-hydroxyazobenzene as orange crystals melting at 146 to 149°. The mother liquor yielded a second crop of crystals melting at 139 to 149° and weighing 20 mg.

Bisulfite-Soluble Fraction of Oxidation 9

The bisulfite-soluble fraction of Oxidation 9 (weighing 2.33 grams) was triturated repeatedly with hot chloroform. An insoluble residue weighing 830 mg. remained. The chloroform solution was applied to a column of acid-washed Magnesol 48 mm. in diameter and 240 mm. in length. Development was accomplished with 1250 ml. of 100:1 petroleum ether-ethanol. The following fractions were obtained by acetone elution of the respective zones.

Zone	Recovered Material, mg.	%
A	660	44.0
B	190	12.7
C	408	27.2
Total	1258	83.9

Fractions A and B were spotted on a paper chromatogram, developed with butanol-aqueous ammonia, and sprayed with bis-diazotized benzidine. The complexity of these fractions was shown by the number of discrete spots which were found; the results are summarized below with an indication of their relative intensities.

Fraction			Color Shown,	
A	B	R_F	Ultraviolet	Spray
*	*	0	blue	tan
	**	.01-.02	purple	brown
*	*	.02-.04	lt. blue	tan
	**	.04-.07	blue	brown
*	*	.08-.11	lt. blue	tan
	****	.14-.22	invisible	red brown
	*	.22-.25	peach	invisible
	*	.37-.45	lt. blue	tan

Fraction B which exhibited a prominent spot corresponding to vanillic acid was treated with petroleum ether and with benzene but no crystalline material could be obtained.

Fraction C was mixed with powdered cellulose and applied in a uniform layer to the top of the previously described powdered cellulose column. Butanol-aqueous ammonia was applied as developer for 45 hours, during which time 90 fractions were automatically cut. Using the techniques described in an earlier section, it was found that the transition from 6-chlorovanillin to vanillin occurred in tube 10. The contents of the tubes were combined and evaporated to dryness according to the following groupings:

Tubes	Recovered Material, mg.	%
1-4 (forerun)	nil	--
5-9	203	49.7
10-20	73	17.9
21-90	31	7.6
50% ethanol eluate	62	15.2
Total	369	90.4

The material recovered from tubes 5-9, melting from 150 to 160°, was triturated repeatedly with hot petroleum ether, leaving an insoluble residue weighing 29 mg. The concentrated and cooled triturate yielded 99 mg. of 6-chlorovanillin as light yellow crystals melting at 166 to 168°.

The yield of 6-chlorovanillin based on chlorite lignin was calculated to be

$$\frac{(203 - 29)}{(7.5)\ (1000)} \times (100) = 2.3\%.$$

The material recovered from tubes 10-20 was triturated repeatedly with hot petroleum ether, leaving an insoluble residue weighing 30 mg. The concentrated and cooled triturate yielded 12 mg. of vanillin melting at 70 to 75°.

The yield of crude vanillin based on chlorite lignin was calculated to be

$$\frac{(73 - 30)}{(7.5)(1000)} \times (100) = 0.6\%.$$

Acid Fraction of Oxidation 9

The acid fraction from Oxidation 9 (weighing 834 mg.) was dissolved in 25 ml. of hot butanol-aqueous ammonia and applied to the previously described powdered cellulose column. Butanol-aqueous ammonia was applied as developer for 66 hours, during which time 132 fractions were automatically collected. Adsorbed material was then removed by elution with 500 ml. of 50% ethanol.

The contents of every tenth tube were evaporated to dryness on the steam bath and, where a significant amount of residue remained, a melting point was taken. In this way, the following groupings of tubes were made and their combined contents were evaporated to dryness:

Tubes	Recovered Material, mg.	%
1-5	139	16.7
6-13	124	14.9
14-30	29	3.8
31-41	73	8.7
42-99	340	40.8
100-132	29	3.5
50% ethanol eluate	150	18.0
Total	884	106.4

The material recovered from tubes 1-5 was dissolved in 95% ethanol, treated with charcoal, filtered, concentrated and cooled to yield 15 mg. of p-phenylazobenzoic acid as bright orange crystals melting at 238 to 240°. Further treatment of the mother liquor in order to obtain more crystalline material was not successful.

No crystalline material could be recovered from the residue obtained from tubes 6-13, 14-30, or 31-41.

The material recovered from tubes 42-99 was triturated repeatedly with hot benzene, leaving an insoluble residue weighing 22 mg. Concentration and cooling of the triturate yielded 231 mg. of vanillic acid melting at 193 to 198°. Evaporation of the mother liquor left a residue melting at 179 to 197°.

The yield of crude vanillic acid based on chlorite lignin was calculated to be

$$\frac{(340 - 22)}{(7.5)\ (1000)} \times (100) = 4.2\%.$$

Phenol Fraction of Oxidation 9

The phenolic fraction of Oxidation 9 (weighing 448 mg.) was triturated repeatedly with hot petroleum ether, leaving an insoluble residue weighing 143 mg. The triturate was concentrated and cooled to yield 128 mg. of p-hydroxyazobenzene as orange crystals melting at 149 to 151°. Concentration of the mother liquor to obtain more crystalline material was not successful.

Recovery of Chlorine as Chloride Ion in Oxidation 9

In Oxidation 6 it was shown that approximately 5% of the chlorine contained in 6-chlorovanillin was converted to chloride ion during the alkaline nitrobenzene oxidation of this compound. It was of interest to make a similar determination of the chloride formation during the oxidation of chlorite lignin itself. Accordingly, the aqueous phase remaining in the continuous extractor after removal of the ether-soluble oxidation products from Oxidation 9 was retained for this purpose.

During three weeks' time a brown gelatinous precipitate appeared. This was removed by filtering and thoroughly washed with water. The filtrate and washings were further acidified with nitric acid and an excess of silver nitrate solution was added. The precipitate was coagulated by heating; it was collected on a tared fritted glass crucible and washed repeatedly with hot dilute nitric acid to ensure removal of silver sulfate. After a final wash with distilled water, it was oven dried to a constant weight of 537 mg. Calculations showed that, assuming the precipitate to contain only silver chloride, approximately 25% of the chlorine content of the chlorite lignin was converted to chloride ion during the oxidation.

SYNTHESIS OF 5- AND 6-CHLOROACETOVANILLONE

In an oxidation of chlorite lignin very similar to those described in this work, Pearl (20) isolated a compound from the phenolic fraction by crystallization from petroleum ether and recrystallization from benzene. It had a melting point of 150 to 151° and gave a positive Beilstein test for halogen. In analogy to the isolation of acetovanillone from the oxidation of other lignin preparations, a chloroacetovanillone was strongly suspected in this case. The elementary analysis supported this theory.

Analysis. Calculated for chloroacetovanillone, $C_9H_9O_3Cl$: C, 53.88; H, 4.52; CH_3O, 15.5. Found: C, 53.79; H, 4.12; CH_3O, 15.4.

The amount of compound isolated was too small to permit further analytical work, although an ultraviolet absorption spectrum was determined.

The only chlorinated acetovanillone reported in the literature is substituted in the side chain methyl group (29); its melting point, 103°, eliminated this possibility of identification.

The synthesis of the two most likely possibilities, 5- and 6-chloroacetovanillone, was consequently undertaken for two reasons: (1) establishment of the physical constants would prove or disprove the identity of the compound isolated by Pearl, and (2) it was hoped that methods might be found for separating the compound from the large amounts of p-hydroxyazobenzene actually isolated in considerable quantity from the phenolic fractions of this work.

The synthesis of the 6-substituted compound was performed first in view of the presence of 6- but not 5-chlorovanillin among the oxidation products.

The first approach to the synthesis of the methyl ketone was through the attempted oxidation of the corresponding carbinol, a compound synthesized by the reaction of methylmagnesium iodide with 6-chlorovanillin. This oxidation was never successful although the following oxidizing agents were used: Fehling solution, acetone and aluminum isopropoxide, neutral permanganate, copper hydrate, silver oxide, and alkaline permanganate.

A second approach to the synthesis, also unsuccessful, consisted of the reaction of methylmagnesium iodide with 6-chlorovanillonitrile acetate which was prepared from 6-chlorovanillin via the oxime (see Figure 1). However, no addition of the Grignard reagent took place, the deacetylated starting material, 6-chlorovanillonitrile, being recovered from the reaction mixture. It is possible that the repetition of this reaction with the free phenol instead of with the acetylated compound might result in the desired addition to the nitrile; such was found to be the case in the somewhat analogous formation of iminoether hydrochlorides from vanillonitrile (30).

The third approach to the synthesis comprised the reaction of diazomethane with 6-chlorovanillin acetate (see Figure 2).

The first time the reaction was attempted, a compound melting at 152 to 154° was obtained in good yield. When this compound was deacetylated a

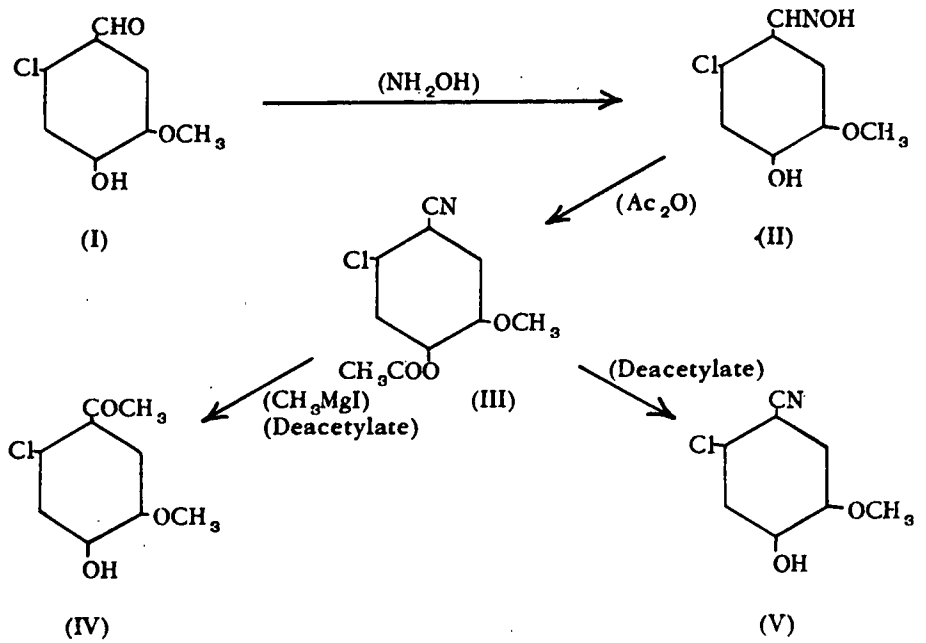

Figure 1

Attempted Synthesis of 6-Chloroacetovanillone via the Nitrile

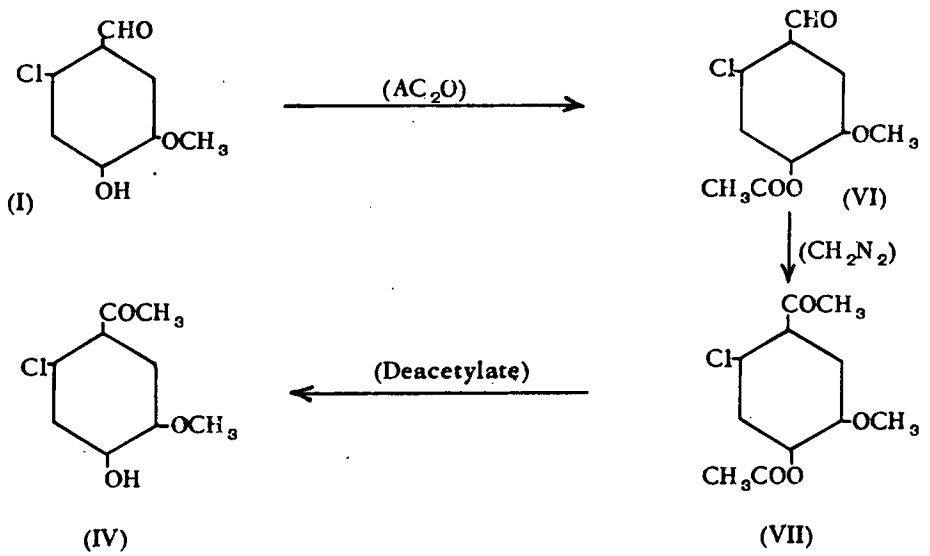

Figure 2

Synthesis of Methyl Ketone by Diazomethane Addition to Aldehyde

product was obtained whose orange color was characteristic of the conjugated structure found in chalcones; the identity of the deacetylated product was subsequently confirmed as being 6,6'-dichloro-4,4'-dihydroxy-3,3'-dimethoxy-chalcone (X) (see Figure 3). However, analyses for carbon, hydrogen, and methoxyl content on the initial product of the diazomethane reaction did not correspond to the chalcone diacetate (IX), but to the chalcone diacetate plus one molecule of water. It is possible that the water was bound as a simple hydrate; however, the lack of any color in the compound and its failure to lose water during prolonged drying over phosphorus pentoxide at elevated temperature (78°) and under aspirator vacuum suggested that the water was actually present in the molecule as the β-hydroxy ketone, 1,3-di(4-acetoxy-6-chloro-2-methoxyphenyl)-3-hydroxy-1-propanone (VIII). An attempt to prepare a triacetate of (VIII) in a pyridine solution with acetic anhydride was not successful but evidently accomplished the dehydration to form the chalcone diacetate (IX), which was subsequently deacetylated to form (X).

A survey of the literature revealed no instance of chalcone formation from the reaction of diazomethane with an aromatic aldehyde. Accordingly, it was decided to run the analogous reaction on vanillin acetate, in which case the expected products are known compounds.

The reaction of diazomethane on vanillin acetate substantiated the theory postulated above, although the reaction did not proceed in exactly the same way. Instead of obtaining a high yield of the chalcone acetate or the corresponding β-hydroxy ketone, a large portion of the starting

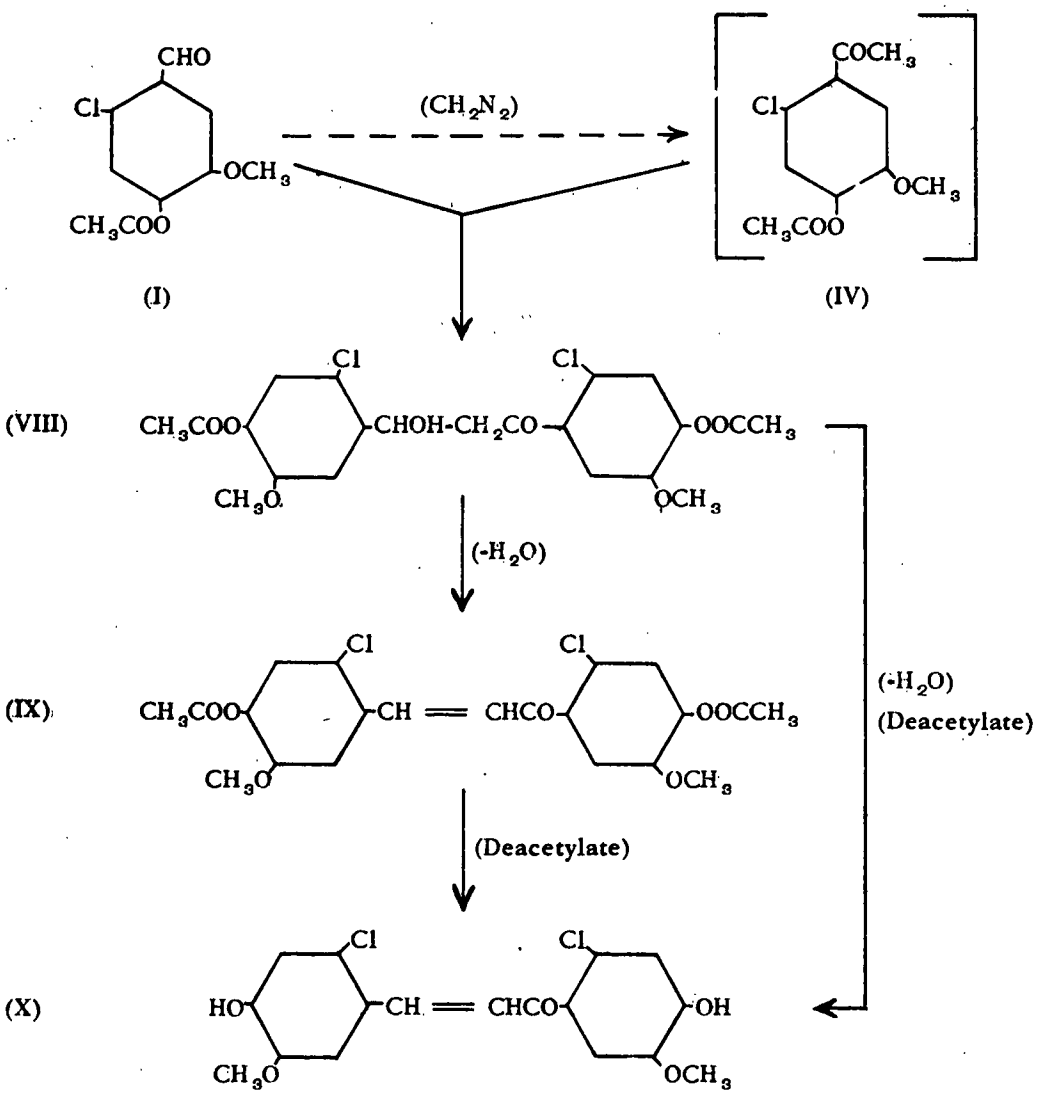

Figure 3

Chalcones from Diazomethane-aldehyde Reaction

material was recovered unchanged. However, acetovanillone was identified as a product in 12% yield and the chalcone was identified by comparison of the paper partition chromatographic behavior of the reaction mixture with known compounds under the action of two different developers (butanol-aqueous ammonia and 2:1 petroleum ether-benzene saturated with water). The R_F values, the appearance under ultraviolet and visible light and, finally, the color produced by a spray of bis-diazotized benzidine were the criteria by which the presence of the chalcone was judged.

In view of the positive evidence for chalcone formation, the reaction of diazomethane on 6-chlorovanillin acetate was repeated with an inversion of the method of addition--i.e., a solution of 6-chlorovanillin acetate was added slowly and with stirring to a solution of diazomethane--in the hope that the excess of the latter might force the reaction to the desired product. Such was not the case; the compound believed to be the β-hydroxy ketone (VIII) was obtained, although a much lower yield resulted.

The desired compound, 6-chloroacetovanillone, was finally isolated in 16% yield from the cleavage of the corresponding chalcone by 20 hours' refluxing with 15% potassium hydroxide. The other fragment of the cleavage, 6-chlorovanillin, was not isolated in this experiment but in another case was recovered in 24% yield merely by warming a 5% sodium hydroxide solution of the corresponding chalcone on a steam bath.

In an attempt to obtain a substantial yield of 6-chloroacetovanillone, the reaction was repeated a third time on a larger scale, using the normal

rather than the inverted addition technique. A sluggish reaction resulted with only a 17% yield of the supposed β-hydroxy ketone (VIII), instead of the 65% yield obtained previously.

When the identity of the synthesized 6-chloroacetovanillone was confirmed and its physical constants did not correspond to those reported by Pearl for the compound which he isolated, the synthesis of the next most likely possibility, 5-chloroacetovanillone, was undertaken by the reaction of diazomethane with 5-chlorovanillin acetate. The 5-chlorochalcone was not isolated as such from the reaction, although there was strong evidence for its presence when one of the reaction products was examined by paper chromatographic techniques. A spot was found whose behavior was very similar to that shown by the corresponding 6-chloro and unchlorinated chalcones. 5-Chloroacetovanillone was isolated by the application of the deacetylated reaction mixture to a column of acid-washed Magnesol. The major zone of the chromatogram yielded a compound which was shown by analytical evidence to be 5-chloroacetovanillone. Again, the physical constants did not correspond to those reported by Pearl for the compound which he isolated.

1-(6-Chloro-4-Hydroxy-3-Methoxyphenyl)-1-Ethanol

Magnesium turnings (7.8 grams, 0.32 mole), were placed in a 2-liter, three-necked flask equipped with a mercury-sealed stirrer, reflux condenser, and dropping funnel. Sixty ml. of absolute ether and 5 grams of methyl iodide were added; reaction was initiated by crushing the magnesium turnings and by warming the reaction flask. An additional 40.5 grams (total 0.32 mole)

of methyl iodide dissolved in 120 ml. of absolute ether were added during 45 minutes with continued stirring for an additional 20 minutes. The reaction mixture was allowed to stand for one hour. Ten grams (0.054 mole) of 6-chlorovanillin dissolved in 1200 ml. of absolute ether were added during 105 minutes, followed by 90 minutes' additional stirring.

After allowing the reaction mixture to stand overnight, 500 ml. of supernatant ether were removed by pipette and replaced by 500 ml. of benzene. With vigorous stirring, 50 ml. of saturated ammonium chloride solution were added dropwise. Addition was stopped when the reaction mixture suddenly cleared up and a gummy solid stopped the stirrer. The ether-benzene layer was decanted and, combined with two ether rinses of the gummy solid, evaporated to yield 10.3 grams of product melting at 118 to 124° (94% yield). Several crystallizations from benzene-petroleum ether yielded buff-colored crystals melting at 128 to 128.5°.

Analysis. Calculated for 1-(6-chloro-4-hydroxy-3-methoxyphenyl)-1-ethanol, $C_9H_{11}O_3Cl$: CH_3O, 15.3. Found: CH_3O, 15.4.

4-Acetoxy-6-Chloro-3-Methoxybenzaldehyde (6-Chlorovanillin Acetate)

To a solution of 15 grams (0.099 mole) of 6-chlorovanillin in 99 ml. of N potassium hydroxide were slowly added, with stirring, 11.5 grams (0.113 mole) of acetic anhydride. A white pasty precipitate was formed immediately. Stirring was continued for 10 minutes. The precipitate was removed from the cooled reaction mixture by filtration and washed with cold water. The yield of crude product, melting from 90 to 93°, was 18.5 grams (82% of theoretical). Two crystallizations from absolute ethanol gave white needles melting at 97 to 97.5°.

Analysis. Calculated for 6-chlorovanillin acetate, $C_{10}H_9O_4Cl$: CH_3O, 13.6. Found: CH_3O, 13.7.

1,3-Di(4-Acetoxy-6-Chloro-3-Methoxyphenyl)-3-Hydroxy-1-Propanone

(A) Normal Addition. A flask containing 6.8 grams of nitrosomethylurea prepared according to Arndt (31) and 75 ml. of ether was cooled to 0° and 20 ml. of a 50% potassium hydroxide solution were added at once. The evolution of diazomethane was allowed to proceed until room temperature had been reached, at which time a water bath was used to distill about three-quarters of the ether. The evolved ether-diazomethane mixture was passed through a water-cooled condenser and introduced beneath the surface of a solution of 8.6 grams of 6-chlorovanillin acetate in 250 ml. of ether kept at 0°. Nitrogen evolution from the reaction mixture proceeded throughout the 45-minute addition of diazomethane and was accompanied by the appearance of a white crystalline mass. This evidence of reaction continued during an additional one-hour period at 0° and for 30 minutes at 20°. The ether and excess diazomethane were then removed by distillation on a water bath. The last traces of ether were removed under vacuum. The crude product, melting from 115 to 145°, weighed 9.0 grams. Crystallization from ethanol raised the melting point to 157 to 158°.

Analysis. Calculated for 1,3-di(4-acetoxy-6-chloro-3-methoxyphenyl)-3-hydroxy-1-propanone, $C_{21}H_{20}O_8Cl_2$: C, 53.52; H, 4.28; CH_3O, 13.2. Found: C, 53.72; H, 4.38; CH_3O, 13.2.

(B) Reverse Addition. An ethereal solution of diazomethane was prepared from 8.6 grams of nitrosomethylurea by the procedure described in the

previous section. To this mechanically stirred and cooled solution were added, during one hour, 2.9 grams of 6-chlorovanillin acetate dissolved in 250 ml. of ether. After 16 hours at room temperature, the supernatant yellow solution was decanted and the ether and excess diazomethane were removed by distillation, yielding 1.3 grams of gummy residue melting at 135 to 147°. The crystals formed during the reaction weighed 0.5 gram and had a melting point of 154 to 156°. A mixed melting point with the corresponding product from the previous section was not depressed.

(C) Second Normal Addition. The conditions used in (A) were repeated on a somewhat larger scale. The diazomethane from 14.6 grams of nitrosomethylurea was passed into a solution of 18.2 grams of 6-chlorovanillin acetate in 750 ml. of ether at 0°. After 4.5 hours, 2.0 grams of the β-hydroxy ketone (VIII) (white crystals melting at 149.5 to 154°) were removed by filtration.

Concentration of the filtrate left an oil which was dissolved in 500 ml. of ether and treated with the same quantity of diazomethane as that described in the previous paragraph. The reaction products yielded 1.1 grams of the β-hydroxy ketone (VIII), as white crystals melting at 149.5 to 153.5°. Combination of this product with the 2.0 grams from the first diazomethane treatment and crystallization from absolute ethanol yielded 2.3 grams of the β-hydroxy ketone (VIII), melting at 157 to 158°.

6,6'-Dichloro-4,4'-Dihydroxy-3,3'-Dimethoxychalcone (6-Chlorinated Chalcone)

Four grams of the β-hydroxy ketone (VIII) were mixed with 30 ml. of 5% sodium hydroxide solution. An orange coloration appeared immediately.

Warming on a steam bath effected complete solution. The reaction mixture was cooled and saturated with carbon dioxide, causing the precipitation of a finely-divided orange solid. This product was extracted from the aqueous solution with chloroform. A crystalline product could not be obtained from the chloroform solution but the use of benzene-petroleum ether as a solvent yielded a crude product melting at 195 to 196°. Recrystallization from the same solvent gave 150 mg. of the 6-chlorochalcone as yellow crystals melting from 196 to 197°. The use of dilute alcohol as solvent gave fine yellow crystals melting from 198.5 to 200°.

Analysis. Calculated for 6,6'-dichloro-4,4'-dihydroxy-3,3'-dimethoxychalcone, $C_{17}H_{14}O_5Cl_2$: C, 55.30; H, 3.82; CH_3O, 16.8. Found: C, 55.38; H, 3.97; CH_3O, 16.5.

The crystals gave a strong Beilstein test for halogen.

The chloroform-extracted solution and the contents of the mother liquor from the crystallization were combined, dissolved in 5% sodium hydroxide, and acidified with sulfur dioxide. An extraction with ether removed material which, after crystallization from benzene, melted from 150 to 180° and weighed 0.75 gram. Purification by crystallization from chloroform-carbon tetrachloride and benzene-petroleum ether yielded 0.17 gram of the 6-chlorochalcone as yellow crystals melting at 194 to 196°.

The ether-extracted bisulfite solution was acidified with 1:1 sulfuric acid and boiled until sulfur dioxide evolution was complete. A precipitate appeared weighing 0.4 gram and melting from 167 to 169°. Crystallization

from benzene raised the melting point to 168 to 169°; no depression was observed in a mixed melting point with an authentic sample of 6-chlorovanillin.

6-Chloroacetovanillone

The crude products of reaction (B) (1.8 grams) and 0.3 gram of the β-hydroxy ketone from reaction (A) were mixed with 80 ml. of 15% potassium hydroxide and refluxed for 20 hours. No perceptible change in the deep orange color was observed. Sulfur dioxide caused the separation of an orange resinous material and a salt which were separated by filtration.

The filtrate, yielded a crop of white crystals during several hours under reduced pressure. The crystals, separated by filtration, had a weight of 0.15 gram and melted from 100 to 105°. Crystallization from dilute alcohol raised the melting point to 109 to 110°. A strong Beilstein test for halogen was obtained.

Analysis. Calculated for 6-chloroacetovanillone, $C_9H_9O_3Cl$: C, 53.88; H, 4.52; CH_3O, 15.5. Found: C, 53.70; H, 4.57; CH_3O, 15.8.

6-Chloroacetovanillone was isolated a second time by a somewhat different procedure. The oil containing all the reaction products of Section (C) except 3.1 grams of the β-hydroxy ketone was only partly soluble in hot 10% sodium hydroxide. After boiling 10 minutes, the cooled alkaline solution was extracted with ether, from which extract were recovered 8.0 grams of alkali-insoluble oil.

The orange solution of alkali-soluble materials was acidified with sulfur dioxide, causing the separation of 4.1 grams of a low-melting orange solid. After several days, the filtrate of the acidified solution yielded a crop of crude 6-chloroacetovanillone as white crystals melting at 98 to 99°. Purification with charcoal and crystallization from dilute alcohol raised the melting point to 109 to 110°; its weight was 40 mg.

A portion of the 4.1 grams of low-melting orange solid described in the previous paragraph was treated with hot benzene and 166 mg. of the benzene-soluble material was applied to a column of acid-washed Magnesol 20 mm. in diameter and 150 mm. in length. The chromatogram was developed with 125 ml. of a mixture of benzene and ethanol (100:1 by volume). The various zones of the column were eluted with acetone to yield the following fractions:

Zone	mg.	Recovered Material %	mg. (second column)
A	43	26.4	140
B	53	31.8	200
C	43	26.4	306
Effluent	11	6.6	
Total	150	91.2	

The results of a similar chromatogram on a larger scale (45 mm. diameter and 220 mm. in length) are also shown on the preceding tabulation.

Fractions A, B, and C were spotted on a sheet of paper and developed with butanol-aqueous ammonia. 6-Chloroacetovanillone was shown to be present only in Fraction C. No appreciable amount of 6-chlorovanillin or the 6-chlorochalcone was present in any fraction. All fractions contained an unidentified material having an R_F of 0.87 to 0.94.

Acetovanillone

Diazomethane, prepared in the manner previously described from 23.2 grams of nitrosomethyurea, was introduced into a solution of 15.0 grams (0.078 mole) of vanillin acetate (32) dissolved in 350 ml. of ether and kept at 0°. There was no apparent reaction at this temperature but allowing the reaction mixture to reach room temperature caused slow gas evolution. The evolution was continuing after 36 hours. The reaction vessel was then warmed slightly on a steam bath for 4 hours. Finally, excess diazomethane and ether were removed by distillation. An oil remained which crystallized after five days. Crystallization from 95% ethanol gave 4.0 grams of a product melting at 67 to 73° and a second crop of crystals weighing 1.2 grams and melting at 65 to 74°. Thus, 34% of the vanillin acetate was recovered unreacted.

Concentration of the mother liquor yielded 8.35 grams of an oil which was dissolved in 5% sodium hydroxide, acidified with carbon dioxide, and extracted with ether. The ether extract, after drying with sodium sulfate, was evaporated to yield an oil weighing 5.2 grams. This product was examined on paper chromatograms using butanol-aqueous ammonia and 2:1 petroleum ether-benzene as developers. Both developers showed the presence of vanillin, acetovanillone, and their corresponding chalcone.

A 1.52-gram portion of the bicarbonate-insoluble product was applied in benzene to a column of acid-washed Magnesol 35 mm. in diameter and 190 mm. in length. Development was accomplished with 250 ml. of 250:1 benzene-ethanol. Extrusion and streaking indicated concentration into three zones which were eluted with acetone to yield the following fractions.

Zone	Recovered Material, mg.	%
A	337	22.2
B	275	18.1
C	712	47.5
Total	1324	87.8

Fraction B, melting at 95 to 105°, was crystallized from dilute ethanol to yield crystals melting at 103 to 107°. This material was rechromatographed under the conditions given above; 76% of the material was concentrated in one zone which, after elution, yielded a product melting at 107 to 111°. Crystallization from water and treatment with charcoal yielded a product melting at 112 to 114°, which did not depress a mixed melting point with an authentic sample of acetovanillone.

An additional 244 mg. of acetovanillone were obtained by dissolving Fraction C in 10% sodium hydroxide, introducing sulfur dioxide, and cooling the acidified solution. Crystals were formed which, after collection by filtration, melted at 111 to 112.5°. The identity of this fraction was checked by a mixed melting point with authentic acetovanillone.

The filtrate from the previous operation was further acidified with 1:1 sulfuric acid and warmed on the steam bath with aeration to remove the sulfur dioxide. The cooled solution was extracted with ether and the dried ether extract was evaporated to yield 0.4 gram of an oil. Trituration of the oil with petroleum ether dissolved 0.3 gram of material which crystallized as crude vanillin melting at 69 to 75°. The product melted at 75.5 to 77° after recrystallization from petroleum ether.

The yield of acetovanillone was calculated to be 12% of the theoretical value.

4,4'-Diacetoxy-6,6'-Dichloro-3,3'-Dimethoxychalcone

In an attempt to form the triacetate of the β-hydroxy ketone (VIII), approximately one-half gram of the compound was dissolved in 2 ml. of hot pyridine and 4 ml. of acetic anhydride were added. After standing for 20 hours, the mixture was poured onto 25 ml. of chopped ice. The oil which formed soon solidified; it melted at 129 to 133°. Several crystallizations from 95% ethanol raised the melting point to 137.5 to 138.5°.

Analysis showed that, instead of an acetylation of the β-hydroxy ketone, a dehydration evidently had taken place forming the chalcone diacetate (IX).

Analysis. Calculated for 4,4'-diacetoxy-6,6'-dichloro-3,3'-dimethoxychalcone: C, 55.64; H, 4.00; CH_3O, 13.7. Found: C, 55.61; H, 4.05; CH_3O, 13.7.

The identity of the diacetate was further confirmed by a deacetylation reaction; 180 mg. of the compound were warmed on the steam bath for 10 minutes with 5 ml. of ethanol and 3 ml. of 5% sodium hydroxide and then neutralized with dilute sulfuric acid. Upon cooling, a crop of yellow needles formed which weighed 150 mg. and melted at 202 to 203.5°. No depression was observed in a mixed melting point with the previously described 6-chlorochalcone (X).

6-Chloro-4-Hydroxy-3-Methoxybenzaldoxime (6-Chlorovanillin Oxime)

To a solution of 3.3 grams (0.059 mole) of potassium hydroxide in 75 ml. of water were added 10 grams (0.054 mole) of 6-chlorovanillin. The mixture was warmed to effect solution; 4.5 grams (0.065 mole) of hydroxylamine hydrochloride were added at once, causing the evolution of heat and the formation of a heavy white precipitate. An additional 4.5 grams (0.08 mole) of potassium hydroxide dissolved in 5 ml. of water were added, causing the formation of a light orange solution.

After 15 minutes, the solution was cooled in an ice bath and acidified with carbon dioxide; the white crystalline product which formed was separated by filtration; it weighed 5.15 grams and melted at 177.5 to 178.5°. Recarbonation of the filtrate gave 3.6 grams melting at 177.5 to 178.5°; this new filtrate in turn was recarbonated, yielding 1.4 grams melting at 176 to 177°. The total yield of 10.15 grams was 94% of the theoretical value. Raiford and Lichty (18) reported a melting point of 178° for 6-chlorovanillin oxime.

4-Acetoxy-6-Chloro-3-Methoxybenzonitrile (6-Chlorovanillonitrile Acetate)

A mixture of 10.15 grams (0.051 mole) of crude 6-chlorovanillin oxime and 16 grams (0.157 mole) of acetic anhydride was refluxed (air condenser) for 20-minutes, after a gentle warming period, during which time the reaction was initiated. The hot solution was poured, with stirring, into 50 ml. of cold water causing the immediate formation of a tan precipitate which, after separation by filtration and air drying, weighed 11.5 grams and melted at 108 to 123°. Crystallization from benzene-petroleum ether did not raise the melting point but the use of dilute

ethanol as solvent yielded 9 grams of crystals melting at 119 to 125°. A recrystallization from absolute ethanol yielded 5 grams of white needles melting at 126.5 to 127°.

Analysis. Calculated for 6-chlorovanillonitrile acetate; $C_{10}H_8O_3ClN$: CH_3O, 13.8. Found: CH_3O, 13.7.

6-Chloro-4-Hydroxy-3-Methoxybenzonitrile (6-Chlorovanillonitrile)

In an attempt to form the methyl ketone by the addition of methylmagnesium iodide to the nitrile acetate, only deacetylation was accomplished.

Magnesium turnings (2.16 grams, 0.089 mole) were placed in a 500-ml. three-necked flask equipped with a mercury-sealed stirrer, reflux condenser, and dropping funnel. Twenty-five ml. of absolute ether containing 2.5 grams of methyl iodide were added. After the reaction had been initiated, 50 ml. of ether containing an additional 12.1 grams (total 0.089 mole) of methyl iodide were added. Stirring was continued for one hour at which time the dropwise addition of a solution of 5.0 grams (0.022 mole) of 6-chlorovanillonitrile acetate in 80 ml. of ether and 80 ml. of benzene was started. This addition was completed in 45 minutes; stirring was continued under reflux for 2 hours and at room temperature for an additional 6 hours.

After the reaction had stood an additional 10 hours, a 1:3 hydrochloric acid-water solution was added dropwise with stirring. The warm reaction mixture became peach-colored and, when 30 ml. of acid had been added, separated into an aqueous phase and an orange ether-benzene phase. An additional 5 ml. of acid were added and the phases were separated. Three

extractions with ether were made on the aqueous phase. The extracts were added to the benzene-ether phase; the combined extracts were washed with water, dried with sodium sulfate, and concentrated, yielding an orange solid. Crystals were obtained from benzene weighing 2.1 grams and melting at 148 to 150°. A second crop of crystals weighed 1.1 grams and melted at 139 to 147°.

These crystalline products were combined, decolorized with charcoal, and crystallized from benzene to yield 1.7 grams of a slightly yellow product melting at 151.5 to 152°. A Beilstein test for halogen was positive, as was a sodium fusion test for nitrogen.

The crude yield of 6-chlorovanillonitrile was 79%.

Analysis. Calculated for 6-chlorovanillonitrile, $C_8H_6O_2ClN$: CH_3O, 16.9. Found: CH_3O, 16.7.

4-Acetoxy-5-Chloro-3-Methoxybenzaldehyde (5-Chlorovanillin Acetate)

A mixture of 15 grams (0.099 mole) of 5-chlorovanillin and 100 ml. of N potassium hydroxide was heated until solution was complete. To the hot solution were slowly added 11.5 grams (0.113 mole) of acetic anhydride. Mechanical agitation was continued for 20 minutes after the completion of the addition as the mixture cooled to room temperature. The oily product crystallized after several hours in an ice bath; it was dissolved in 95% ethanol, treated with charcoal, and crystallized from dilute ethanol to yield a product melting at 55 to 61°. Crystallization from low-boiling petroleum ether yielded 7.5 grams of white crystals melting at 61 to 63°. A second crystallization from this solvent raised the melting point to 63 to 64°. The product exhibited a strong Beilstein test for halogen.

Analysis. Calculated for 5-chlorovanillin acetate, $C_{10}H_9O_4Cl$: CH_3O, 13.6%. Found: CH_3O, 13.8%.

5-Chloroacetovanillone

A solution of 7.15 grams (0.0313 mole) of 5-chlorovanillin acetate in 250 ml. of c.p. ether was cooled to 0°. Diazomethane, prepared in the previously described manner from 6.8 grams of nitrosomethylurea, was introduced into the 5-chlorovanillin acetate solution during 45 minutes. No gas evolution was apparent during this time but was perceptible when the reaction mixture was allowed to warm to room temperature. After standing for several days, the yellow color was dissipated but removal of the ether did not yield a crystalline product.

The oily product was dissolved in 250 ml. of c.p. ether at room temperature and the diazomethane generated from 38 grams of nitrosomethylurea was introduced into the solution. Gas evolution was apparent for 24 hours. The reaction mixture was allowed to stand at room temperature for three weeks during which time the ether evaporated, leaving a yellow oil weighing 7.4 grams.

The oil could not be crystallized from 95% ethanol or benzene; neither could a chromatographic separation be effected on acid-washed Magnesol using benzene-ethanol or petroleum ether-ethanol as developers.

An aliquot of the yellow oil weighing 1.96 grams was boiled briefly with 5% sodium hydroxide, causing a portion of the oil to dissolve. The insoluble portion was dissolved in ether and extracted twice with 5% sodium hydroxide.

The combined alkaline solutions, deep orange in color, were saturated with sulfur dioxide and extracted with ether; the combined ether extracts, after being washed with water and dried over sodium sulfate, yielded 1.0 gram of an oil which could not be crystallized from benzene.

A portion of this oil (961 mg.) was dissolved in benzene and applied to a column of acid-washed Magnesol 38 mm. in diameter and 240 mm. in length. Development was accomplished with 500 ml. of 100:1 benzene-ethanol. The fractions recovered by acetone elution of the zones are listed below.

Zone	Recovered Material, mg.	%
A	109	11.3
B	187	19.4
C	26	2.7
D	189	19.6
E	398	41.5
Total	909	94.5

Fraction E melted at 122 to 125°. Its analysis and the analogy to the vanillin-diazomethane reaction showed it to be 5-chloroacetovanillone in 23% of the theoretical yield (based on 5-chlorovanillin acetate). It was dissolved in 95% ethanol, treated with charcoal, and crystallized from dilute ethanol to yield 340 mg. of white crystals melting at 123 to 125°. A second crystallization from dilute ethanol raised the melting point to 124.5 to 125°.

Analysis. Calculated for 5-chloroacetovanillone, $C_9H_9O_3Cl$: C, 53.88; H, 4.52; CH_3O, 15.5. Found: C, 54.01; H, 4.53; CH_3O, 15.4.

An additional 110 mg. of crude 5-chloroacetovanillone, melting at 118 to 121°, was obtained from Fraction D by crystallization from benzene-petroleum ether; the product melted at 121 to 123° after recrystallization.

A paper chromatogram using butanol-aqueous ammonia as a developer was run on all fractions obtained from the reaction. Fraction D from the acid-washed Magnesol column and the 685 mg. fraction from the alkali-insoluble portion of the original aliquot exhibited a spot at R_F 0.09 to 0.15, which showed a brilliant yellow fluorescence when viewed under visible light while in a basic condition. This behavior corresponded to that shown by the analogous 6-chloro and unchlorinated chalcones previously described and suggested that 5,5'-dichloro-4,4'-dihydroxy-3,3'-dimethoxychalcone was contained in these fractions. An unidentified spot at R_F 0.52-0.59, which gave a brilliant red coloration when sprayed with bis-diazotized benzidine, was found in Fraction A from the acid-washed Magnesol column.

THE USE OF ANION-EXCHANGE RESINS IN SEPARATING VANILLIN FROM VANILLIC ACID

When orientative Oxidations 1 and 2 showed that vanillin and vanillic acid were not being quantitatively separated by the methods used, a search was made for a new technique by which this might be accomplished. The use of anion-exchange resins appeared to hold promise, inasmuch as compounds are adsorbed on the resin bed solely on the basis of the relative acidities, as contrasted to the conflicting influence of partition coefficients when dealing with the extraction of ether solutions with various aqueous solutions.

When working with pure compounds, the method proved to be quite successful; in run 3 a mixture of vanillin and vanillic acid was separated with recoveries of 98.5 and 93.4% of the respective compounds. However, the application of the technique to the products of an alkaline nitrobenzene oxidation of chlorite lignin (Oxidation 3) was only partially successful. A phenolic fraction of sufficient purity was obtained to permit the isolation of crystalline 6-chlorovanillin but, since the acidic materials retained on the column did not yield vanillic acid by any of the separation methods applied, the anion-exchange method was abandoned.

Four weakly basic ion-exchange resins were investigated for their suitability in separating vanillin from vanillic acid. They were Duolite A-2 and A-3 (Chemical Process Company), Deacidite (Permutit Company), and Ionac A-300 (American Cyanamid Company). Resin beds of approximately 125 mm. depth were contained in glass tubes of 18 mm. diameter. A cotton plug prevented passage of resin particles into the outlet tube which led from the bottom of the cylinder to a point above the upper level of the bed. In this way, the solution was made to cover the resin bed at all times. Solutions were applied to the column from a 50-ml. burette. Resins were extracted with hot alcohol to remove alcohol-soluble materials which might otherwise contaminate compounds placed on the column. Regeneration was accomplished by treatment with a volume of N sodium hydroxide four times greater than the volume of resin, followed by distilled water until a neutral effluent was obtained. The washing was actually accomplished by the overnight passage of 7 to 8 liters of distilled water.

A preliminary experiment with Duolite A-2 showed that the particular sample used was not suitable for use with alcoholic solvents because of contamination from prior use with a material which could not be removed by repeated alcohol extraction. The other three resins were tested as follows.

Run 1

Fifty ml. of 1:1 ethanol-water were passed through the regenerated column, followed by 25 ml. of a 1:1 ethanol-water solution containing 1.125 grams of vanillin and 0.975 gram of vanillic acid. (Ethanol solutions were used to reduce the volume of solvent required to apply the compounds to the column.) Elution was accomplished by an additional 300 ml. of 1:1 ethanol-water. The various fractions of effluent were collected and evaporated to dryness yielding the results tabulated below.

Fraction, ml.	Duolite A-3		Deacidite		Ionac A-300	
	mg.	%[1]	mg.	%	mg.	%
50	34	3.2	41	3.6	43	3.8
50	654	61.2	473	45.7	288	28.4
50	252	83.6	212	64.6	163	43.8
50	81	91.9	70	70.8	69	50.0
50	14	92.1	40	74.3	74	56.6
100	16	93.5	63	80.0	55	61.5

[1]% = Aggregate wt./wt. of vanillin applied to column.

In view of the more complete removal of vanillin from Duolite A-3, it was the only resin from which vanillic acid was isolated. This was accomplished by passing 5% sulfuric acid in 1:1 ethanol-water through the resin bed until the effluent was acidic. Evaporation of the ethanol yielded 850 mg. of crude vanillic acid melting from 203 to 206°; this represents 88% of the acid applied to the column.

The combined effluent prior to acid washing yielded a solid whose chloroform-soluble portion contained 600 mg. of crude vanillin melting at 79.5 to 81.5°. (Unfortunately, a crude vanillic acid containing some quantity of inorganic salt was used in the experiments herein described. Thus, the vanillin was contaminated and the actual quantity of vanillic acid applied to the column was less than the value given.)

Run 2

A second run was made on Duolite A-3 using a higher alcohol concentration (4:1) in order to obtain higher vanillin recovery in a smaller volume of effluent. Melting points of the various effluent cuts showed, however, that vanillic acid was not being held by the column but was appearing in substantial amounts long before the vanillin had left the column.

Run 3

A third separation was made on Duolite A-3 using 1:1 ethanol-water as solvent and using N sodium hydroxide to remove the adsorbed vanillic acid. Sixty ml. of the resin, regenerated and prewashed with solvent as described above, were contained in a 21.5 mm. diameter tube. A solution containing 5 grams of vanillin and 2.5 of vanillic acid in 180 ml. of solvent was applied at a rate of 2 to 2.5 ml. per minute and followed by 370 ml. of solvent. The distribution of vanillin in the effluent is shown below. Each effluent cut was 50 ml. in volume.

Effluent Cut	Weight, mg.
1	2
2	62
3	1340
4	1797
5	1144
6	275
7	120
8	87
9	56
10	20
11	24

The 550 ml. of effluent contained 4.927 grams of crude vanillin (98.5% recovery). Crystallization from water yielded 4.023 grams of vanillin, melting at 81 to 82° (80.5% recovery); a second crop of crystals weighing 0.283 gram and melting at 79.5 to 81° accounted for an additional 5.6% of the vanillin applied to the column.

Vanillic acid was removed from the column by elution with 130 ml. of N sodium hydroxide. This volume was determined by periodically acidifying samples of effluent until no precipitation was observed. Crystallization of the combined acidified effluent yielded 2.337 grams of vanillic acid melting at 205 to 207° (93.4% recovery).

QUALITATIVE SEPARATIONS BY PAPER PARTITION CHROMATOGRAPHY

Paper partition chromatography proved to be a useful tool in working with lignin oxidation products. It was particularly helpful when used in conjunction with column chromatograms by indicating which zones of the column contained significant concentrations of the materials under investigation.

In this work, the paper partition chromatographic technique was first applied to the bisulfite-soluble fraction of Oxidation 3 to determine whether or not vanillin and 5-chlorovanillin were present in addition to the 6-chlorovanillin which had been isolated. Small samples of each of these compounds, as well as the unknown mixture, were dissolved in 95% ethanol. These solutions were applied dropwise from capillary tubes to points at one end of 24-inch strips of Whatman No. 1 filter paper. After evaporation of the alcohol, the deposited material was carried down the sheet by a developer into which the upper end of the paper was immersed. The operation was carried out in a closed glass cylinder, the atmosphere of which was saturated with the developing agent. After a period of development the sheet was removed, the developer evaporated from the sheet, and a reagent applied as a spray. The positions of the respective compounds were shown by the appearance of color.

A saturated solution 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid was used initially as a spray reagent; this caused the formation of an orange color with each of the three compounds in question. However, it was subsequently replaced by a butanol solution of aniline hydrogen phthalate (prepared by mixing 0.093 gram of aniline and 1.66 grams of phthalic anhydride in 100 ml. of water-saturated butanol). With this reagent, no secondary spray of sodium bicarbonate solution was necessary to neutralize the acidic residue of the former reagent nor was there any background color apparent on the paper. Of even greater importance was the distinctive green fluorescence exhibited by the vanillin spot when viewed under ultraviolet light; this permitted its differentiation from the other two compounds

which showed no fluorescence. By visible light, when sprayed with aniline hydrogen phthalate, the compounds exhibited a bright yellow color which was accentuated by ten minutes' heating in a 105° oven.

Three ineffective developing solutions were investigated and discarded before a satisfactory combination could be found. In each case, the atmosphere of the cylinder was exposed to both the developer and the aqueous phase with which the developer was saturated; this was accomplished by hanging strips of filter paper from troughs containing the two phases and allowing equilibration of the atmosphere before introducing the sample.

Petroleum ether saturated with water was the first developer attempted, inasmuch as it had been shown to be successful in separating vanillin, syringaldehyde, and p-hydroxybenzaldehyde (33, 34). However, very diffuse spots were obtained, especially in the case of 6-chlorovanillin. The same diffusion problem was encountered when the petroleum ether was saturated with an ethanol-water mixture (5:1 by volume). When a pyridine-butanol-water mixture was used (3:10:3 by volume), all compounds moved parallel down the strip with the solvent front.

Finally, butanol saturated with 2% aqueous ammonia was used in view of its successful separation of vanillin and ethylvanillin (35). A good separation was obtained, yielding R_F values of 0.36, 0.46, and 0.66 for vanillin and its 5- and 6-chloro derivatives, respectively. When a mixture of the three compounds was developed in this way, the vanillin and 5-chloro-vanillin spots were contiguous and difficult to differentiate under visible

light but exposure to ultraviolet light plainly showed their positions. The fluorescent phenomenon was not permanent; considerable fading occurred over a month's time.

The techniques just described were then applied to the separation of vanillic acid and its 5- and 6-chloro- derivatives from one another. Neither aniline hydrogen phthalate nor 2,4-dinitrophenylhydrazine produce spots with these acidic compounds. Instead, a solution of ammoniacal silver nitrate was first used but was superseded by a more satisfactory spray made up of bis-diazotized benzidine. The ammoniacol silver nitrate solution was prepared by mixing equal parts of 0.1 N silver nitrate and 5 N ammonium hydroxide; following spraying, careful heating in a 105° oven was required to bring out the spots in brown or reddish-brown shades.

The bis-diazotized benzidine solution, used by Leopold for coupling with phenolic compounds, was prepared in the following way.

Two solutions were prepared and stored in the refrigerator. Solution A contained 1.25 grams of benzidine in a solution of 250 ml. of water containing 4 ml. of concentrated hydrochloric acid; Solution B contained 2.5 grams of sodium nitrite dissolved in 250 ml. of water. Immediately before use, equal portions of Solutions A and B were mixed. A small amount of powdered sodium carbonate was then added to make the solution alkaline for coupling. The spray reagent must be used while cold as it rapidly decomposes at room temperature.

A number of different developers was investigated for their ability to separate vanillic acid and 6-chlorovanillic acid. The only promising one found was butanol saturated with water used on paper buffered to pH 7.5. Other developers which produced no separation were butanol-aqueous ammonia, butanol-water on unbuffered paper, pyridine-butanol-water (3:10:3), methyl ethyl ketone-water, butanol-ethanol-7 N ammonia (4:12:2), 2:1 or 4:1 chloroform-95% ethanol with 1% of 90% formic acid, and 3:1 phenol-water with 1% of 90% formic acid.

Table IV lists the chromatographic characteristics of a number of compounds when spotted on paper and developed in several different systems, including butanol-aqueous ammonia, butanol-water, and petroleum ether-benzene. Part of this compilation includes some of the compounds recently listed by Leopold (14) as alkaline nitrobenzene oxidation products of sprucewood. Generally speaking, agreement was obtained with the chromatographic behavior which he reported. There is one notable exception; 5-carboxyvanillic acid (and its dihydrate) showed a much lower R_F than was reported by Leopold (0.04 vs. 0.67). Leopold does not report the source of his sample; the sample used in this work was prepared by Pearl in three different ways--namely, by the oxidations of 5-carboxyvanillin, 5-formylvanillic acid, and 5-formylvanillin. The lack of agreement in R_F values throws some suspicion of the authenticity of Leopold's sample.

The listings in Table IV demonstrate some of the separations which may be achieved in the identification of mixtures of compounds to be found in this work. It was found that considerable variation in R_F values might occur

from one run to the next because of differences in developer composition, the pH of buffered paper and, in some cases, the other components of the mixture. For this reason, the R_F values have only a limited diagnostic value; the behavior of an authentic sample on the same chromatogram is the only true criterion of identity. This is also necessary for a comparison of the colors exhibited under visible or ultraviolet light or when sprayed.

TABLE IV

THE CHROMATOGRAPHIC BEHAVIOR OF SOME PHENOLIC COMPOUNDS

Compound	R_F
Developer I. Butanol saturated with 2% aqueous ammonia, unbuffered paper	
Dehydrodivanillic acid	0.00
5-Carboxyvanillic acid	0.02
5-Formylvanillic acid	0.03
Protocatechuic acid	0.07
Vanillic acid	0.14
6-Chlorovanillic acid	0.14
4,4'-Dihydroxy-3,3'-dimethoxy-chalcone	0.13-0.37
5-Carboxyvanillin	0.31
Protocatechualdehyde	0.32
Syringaldehyde	0.36
Vanillin	0.36
6,6'-Dichloro-4,4'-dihydroxy-3,3'-dimethoxychalcone	0.43-0.55
5-Chlorovanillin	0.46
6-Chloroprotocatechualdehyde	0.47
Acetovanillone	0.60
6-Chloroacetovanillone	0.62
6-Chlorovanillin	0.65
p-Hydroxybenzaldehyde	0.65
Pyrocatechol	0.85
p-Hydroxyazobenzene	0.88
Guaiacol	0.92

TABLE IV (Continued)

THE CHROMATOGRAPHIC BEHAVIOR OF SOME PHENOLIC COMPOUNDS

Compound	R_F	
Developer II. Butanol saturated with water, paper buffered to pH 7.5		
		Leopold (14)
Dehydrodivanillic acid	0.04	0.06
5-Carboxyvanillic acid	0.09	0.67
5-Carboxyvanillic acid dihydrate	0.09	
5-Formylvanillic acid	0.31	0.31
Vanillic acid	0.42	0.48
6-Chlorovanillic acid	0.48	
Developer III. 2:1 Petroleum ether-benzene saturated with water, paper buffered to pH 9 with saturated borax solution		
Vanillin	0.14	
Acetovanillone	0.35	
4,4'-Dihydroxy-3,3'-dimethoxy-chalcone	0.04	

Leopold recommended the use of a Swedish handmade filter paper, Munktell OB, as being superior to Whatman No. 1. Several comparisons were made on the closest available grade of Munktell paper (No. 1), with no appreciable difference in behavior being apparent.

The buffered paper, unless otherwise indicated, was prepared by dipping a sheet of paper in a container of McIlvain's standard buffer solution (citric acid and disodium phosphate) and allowing the sheet to air dry. It was then treated in the usual manner.

6-CHLOROVANILLIC ACID

A small quantity of 6-chlorovanillic acid was required for use in a study of the paper partition chromatography of vanillic acid and its 5- and

6-chloro derivatives. The compound had previously been prepared from 6-chlorovanillin via the oxime and acetoxynitrile by Raiford and Potter (36). In view of the success of silver oxide oxidations of vanillin and 5-chlorovanillin to the corresponding acids (37), the same method was applied to 6-chlorovanillin.

Silver oxide was prepared from equimolar amounts (0.05 mole) of silver nitrate (8.50 grams) and sodium hydroxide (2.10 grams), each in 25% aqueous solution. The thoroughly washed silver oxide (0.025 mole) was suspended in 50 ml. of water and 10 grams (0.25 mole) of sodium hydroxide were added. The temperature rose to 43° and 4.69 grams (0.025 mole) of 6-chlorovanillin were added. The reaction vessel was heated until 75° was reached, at which time a spontaneous temperature rise was observed and silver separated. The temperature was raised to 90° and then allowed to drop to room temperature. The reaction mixture was filtered on a sintered glass funnel and the precipitate was washed repeatedly with water. The filtrate, light yellow in color, was acidified with 1:1 sulfuric acid and the white precipitate filtered and washed with water. The 4.15-gram precipitate (82% of theoretical yield) had a melting point of 204 to 206°; Raiford and Potter (36) reported a melting point of 207°.

An earlier experiment with only one half mole of silver oxide produced a crude acid in much lower yield.

QUANTITATIVE SEPARATIONS BY PAPER PARTITION CHROMATOGRAPHY

In addition to the qualitative separation of mixtures as spots, some work was done using a somewhat heavier filter paper (Whatman 3M) to which

a significant quantity of material could be applied from a tube drawn to a fine point. The technique was first tried with known quantities of vanillin and 6-chlorovanillin with indifferent success. It was not possible to prepare a concentrated solution of the materials in alcohol because of crystallizing tendencies. Since the application is by nature a tedious one (repeated application of the solution with allowance for solvent evaporation between applications), the vanillin and 6-chlorovanillin were dissolved in a small amount of acetone. However, the rapid diffusion of the acetone, carrying with it the dissolved compounds, resulted in the buildup of two lines of crystals about 5 mm. on either side of the desired line. Consequently, the separation of the compounds in the developed chromatogram was poor.

Somewhat greater success was had with the crude mixture of acidic materials as was described in Oxidation 4. It was possible to obtain a fairly high concentration of the fraction in warm butanol, which was applied to the sheet in this form. Examination of the sheet under visible and ultraviolet light after development with butanol-aqueous ammonia showed five discrete bands. A strip cut down the center of the chromatogram was sprayed with bis-diazotized benzidine and revealed two more bands. The chromatogram was cut into 16 strips corresponding to the observed bands and each strip was eluted according to the Dent technique (24) with 50% ethanol; the eluate was collected and evaporated in tared Petri dishes, thereby giving the weight distribution pattern of the various bands.

The Dent procedure comprises placing the end of the strip of paper between two microscope slides; the opposite ends of the slides are dipped in the eluting agent which advances between the slides and down the strip to be collected as it drips from the bottom of the paper. The quantitative nature of the method was checked by eluting 97% of the vanillin on a 7.5 cm. by 9 cm. strip of paper in 6 hours with 50% ethanol.

Elution of the acid-fraction chromatogram showed a fairly uniform distribution of material along the length of the sheet with the exception of the vanillic acid--6-chlorovanillic acid area. The use of ultraviolet light is somewhat deceptive in band location, inasmuch as some strongly fluorescent materials may give the false indication of being present in significant amounts.

POWDERED CELLULOSE COLUMN CHROMATOGRAPHY

Powdered cellulose columns have been used as a quantitative extension of the compound separations observed on sheets in paper partition chromatography. Most of the work in the literature has been done with sugars and sugar derivatives but the use of this technique in separating aldehydic products of the alkaline nitrobenzene oxidation of wood has recently been reported by Leopold (14). Generally, a series of arbitrary eluate fractions are taken from the bottom of the column, thereby obtaining the separate components of the mixture as they move down the column at different rates. Numerous attempts have been made to apply the method to the fractions obtained from the oxidation of chlorite lignin and, although in some cases it has been found extremely useful, there is considerable evidence to show

that the behavior of a column of cellulose is frequently quite different from that which is observed on a strip of paper using the same developer.

The most successful application (see Oxidations 4, 8, and 9) will be discussed first. Vanillin and 6-chlorovanillin are easily separated as spots on a strip of paper using butanol-aqueous ammonia as a developer; average R_F values of 0.36 and 0.66, respectively, indicate the magnitude of the separation. Because they are colorless compounds, a search was made for a dye which would have an intermediate R_F value, thereby marking the point at which eluate receivers should be changed. Methyl orange was judged to be suitable in view of its R_F value of 0.54 when spotted on a sheet.

However, when a known mixture of vanillin and 6-chlorovanillin with a trace of Methyl orange was applied to a column, the behavior was quite different. First, the separation between zones of the two major components was very slight if actually existent. Instead, the vanillin zone appeared to be immediately following the 6-chlorovanillin zone, rather than being separated as the widely different R_F values would have indicated. Second, the Methyl orange, as a broad band, actually led both materials down the column, overlapping somewhat into the 6-chlorovanillin zone. Consequently, the use of a marker dye was discarded and the eluate was tested periodically by collecting a drop on a filter paper and adding a drop of 2,4-dinitrophenylhydrazine reagent. Not only did this serve to identify the presence of a carbonyl-containing compound but a color difference served to distinguish between vanillin and 6-chlorovanillin.

Vanillin and 6-chlorovanillin mixtures were successfully applied to cellulose columns by thoroughly mixing a portion of powdered cellulose with the sample and distributing it uniformly as a band at the top of the column; separations were equally satisfactory whether the mixture was wet with acetone, which was subsequently evaporated to distribute the materially uniformly on the fibers, or whether the mixture was simply finely ground to insure small particle size.

Turning now to the application of the powdered cellulose column technique to the separation of the acid fraction, a somewhat different behavior was observed. When the acid fraction was applied as a narrow band to the top of the column either by dry mixing with powdered cellulose or by the evaporation of an acetone solution of the acidic material onto cellulose, a very poor chromatogram was obtained. Although a considerable number of discrete bands were visible, either under visible or ultraviolet light, there was a serious skewing of the pattern--deep "stabs" made impossible the isolation of single bands by the collection of eluate fractions.

The skewing and "stabbing" problem was satisfactorily solved by applying the acidic fraction in a fairly dilute solution to the top of the column. As development proceeded, discrete bands were formed in very uniform zones. This behavior was quite unexpected, being more characteristic of adsorption chromatography than of partition chromatography; the latter technique usually is the more successful, the narrower the area of application.

Although the acid fraction exhibited a large number of discrete bands (eight to nine) when viewed under visible and ultraviolet light, the normal coning or doming behavior of chromatographic columns would have rendered useless the collection of eluate fractions as a means of separating closely situated narrow bands. Accordingly, some work was done on the extrusion of powdered cellulose columns which had been formed in tapered glass tubes. It was found to be entirely feasible. No vacuum could be used in the formation of the column or during development because of the strong tendency of the fibrous mass to pull away from the side of the tube leading to serious channeling. Although the usual technique of band location on extruded columns by streaking was satisfactory, some difficulty was experienced in carving away the contaminated portion of the column. Cutting a column under ultraviolet light was found to be more desirable, not only for this reason but also from the standpoint of compensating for doming or coning of zones in otherwise colorless bands. The section thus obtained was then eluted with acetone.

Using this technique, an aliquot of the acid fraction was separated into nine different zones; however, only two of these contained more than 3% of the material applied to the column and none yielded a pure compound. The melting point of the material eluted from the largest zone (190 to 197°) indicated that vanillic acid was being concentrated in that area.

A significant difference in the behavior of cellulose as compared with acid-washed Magnesol is in its adsorptive power toward the materials present on the column. Although acetone is an excellent eluting agent for

recovering materials from Magnesol, its use on cellulose is not satisfactory. Thus, when acetone was used to elute the material from the powdered cellulose sections in the acid fraction separation just described, only 57% of the material was recovered; the cellulose had a distinct brown coloration despite exhaustive elution with acetone. Subsequent work showed 50% ethanol to be a much more satisfactory eluting agent.

An excellent example of the difference in behavior between a sheet of paper and powdered cellulose in a column was the case of vanillin, acetovanillone, and the corresponding chalcone. When these three compounds were developed with butanol-aqueous ammonia as spots on a sheet of paper, they were widely separated and easily distinguished having R_F values of 0.44, 0.60, and 0.13-0.37, respectively. However, when an attempt was made to convert the separation to a quantitative one on a powdered cellulose column, the chalcone formed a diffuse band extending from the solvent front all the way back to its predicted position near the top of the column. This behavior was observed despite such changes in procedure as extremely light loading and extensive predevelopment of the column to insure equilibration between developer and adsorbent.

A brief study was made of some of the variables involved in the separation of vanillin and 6-chlorovanillin on a powdered cellulose column, using butanol-aqueous ammonia as developer. In all cases, the separation between the two zones was much less than would be predicted from the behavior of spots on a sheet and the zones were quite diffuse, despite very light loading. The conditions studied were: (1) method of loading (dry mixing

with cellulose), evaporation from a solution onto a portion of cellulose powder, application of material while in solution in a small portion of developer, and adsorption of material onto a piece of filter paper cut to fit the top of the column, (2) starting the run on a dry column as compared with a column prewet with developer, and (3) changing the concentration of ammonia in the developer.

ULTRAVIOLET ABSORPTION SPECTRA OF SYNTHESIZED COMPOUNDS

Figures 4 and 5 present the ultraviolet absorption spectra exhibited by vanillin, acetovanillone, and their 5- and 6-chloro derivatives, as well as that of the unknown phenolic compound found by Pearl and described in an earlier section of this work.

All data were taken in ethanolic solution with the exceptions of vanillin and 5-chlorovanillin which were dissolved in dioxane.

It is seen that the replacement of the side chain hydrogen in vanillin by a methyl group to form acetovanillone causes the spectrum to be shifted slightly to longer wavelengths; the reduced specific extinction values are to be attributed to the increase in molecular weight.

Similarly, the substitution of chlorine into a molecule does not produce any profound changes. The increased molecular weight reduces the specific extinction values of the entire curve. The 6-chloro substituent appears to induce a greater shift in maxima than does the 5-chloro group.

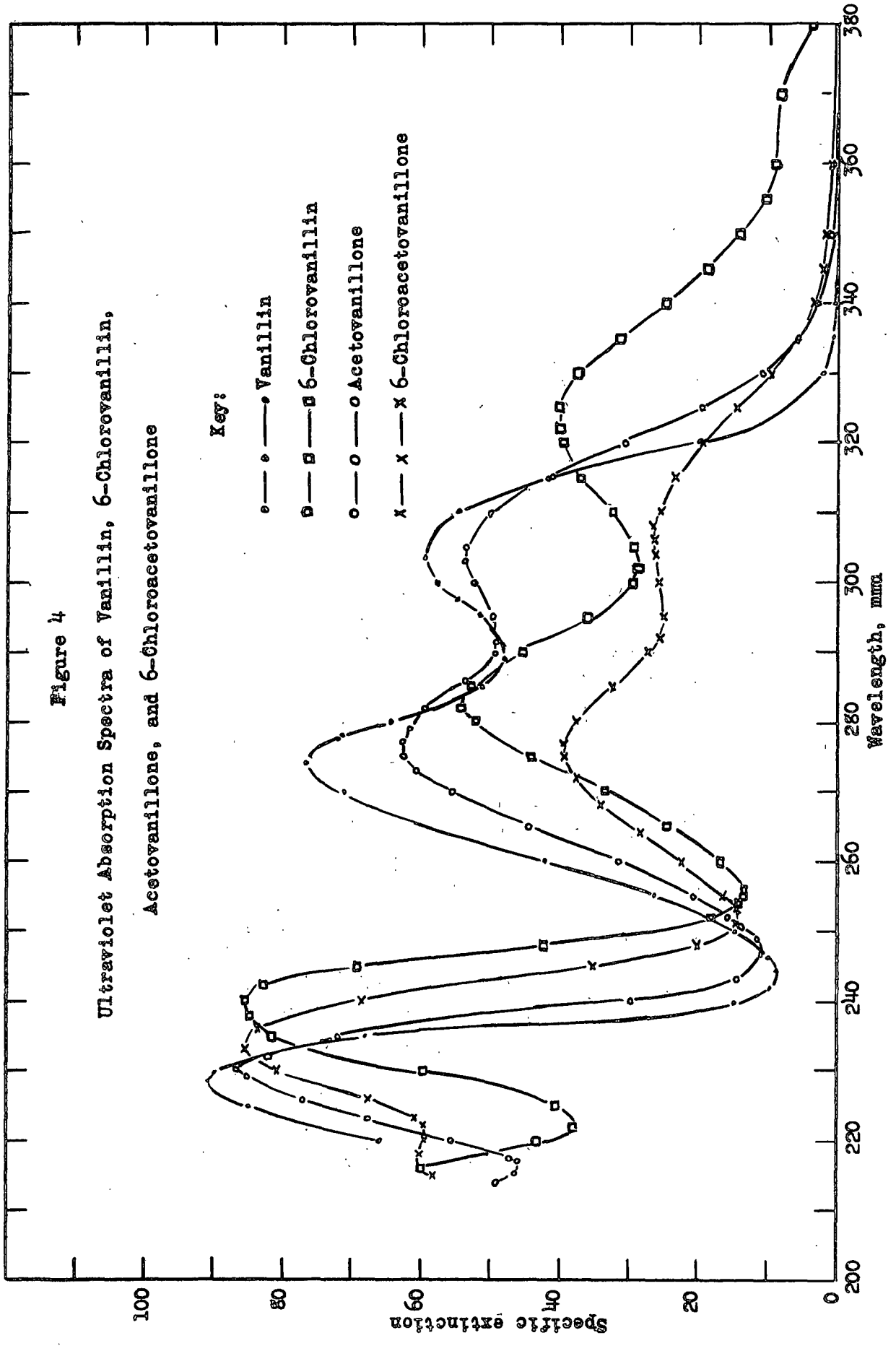

Figure 4

Ultraviolet Absorption Spectra of Vanillin, 6-Chlorovanillin, Acetovanillone, and 6-Chloroacetovanillone

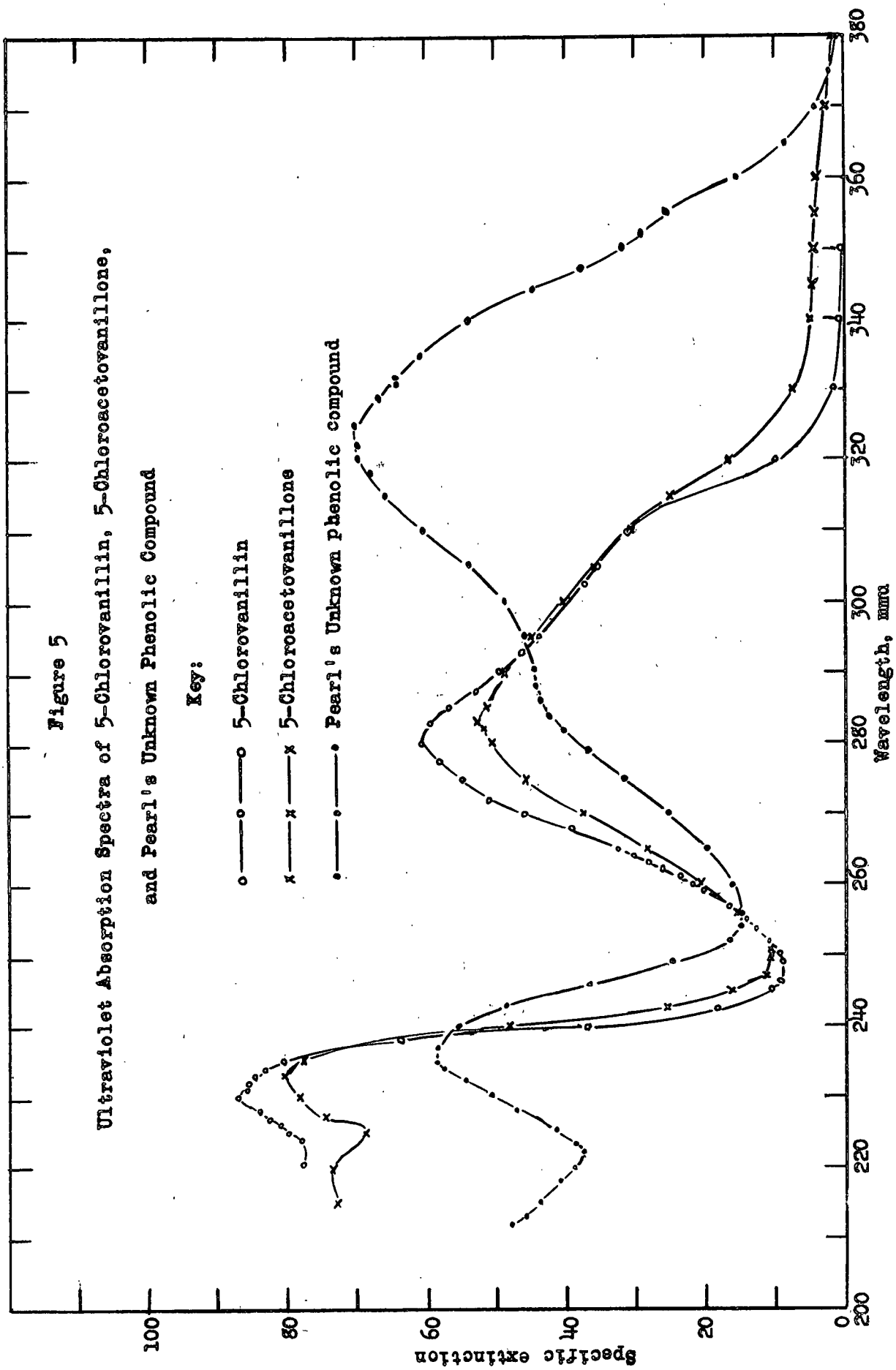

Figure 5

Ultraviolet Absorption Spectra of 5-Chlorovanillin, 5-Chloroacetovanillone, and Pearl's Unknown Phenolic Compound

Pearl's compound shows a maximum at 236 mmu, a minimum at 255 mmu, and a point of inflection at 290 mmu which correspond generally to the characteristics of the other compounds shown. The maximum at 323 mmu, however, is foreign and must be attributed to an entirely different configuration.

Figure 6 presents the ultraviolet absorption spectra of 1,3-di(4-acetoxy-6-chloro-3-methoxyphenyl)-3-hydroxy-1-propanone, 6,6'-dichloro-4,4'-dihydroxy-3,3'-dimethoxychalcone, and 4,4'-diacetoxy-6,6'-dichloro-3,3'-dimethoxychalcone.

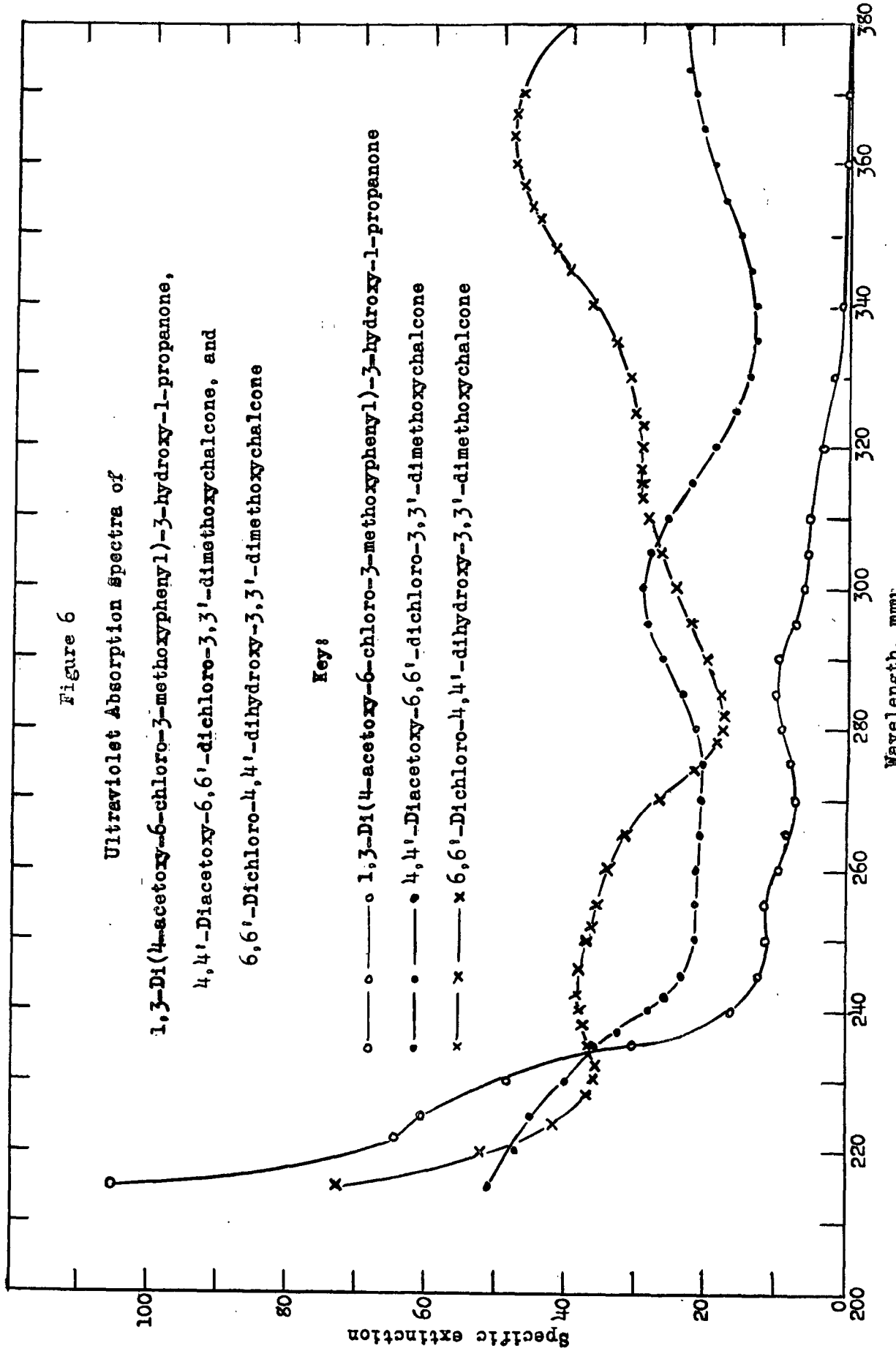
Figure 6
Ultraviolet Absorption Spectra of
1,3-Di(4-acetoxy-6-chloro-3-methoxyphenyl)-3-hydroxy-1-propanone,
4,4'-Diacetoxy-6,6'-dichloro-3,3'-dimethoxychalcone, and
6,6'-Dichloro-4,4'-dihydroxy-3,3'-dimethoxychalcone
Key:
o——o 1,3-Di(4-acetoxy-6-chloro-3-methoxyphenyl)-3-hydroxy-1-propanone
•——• 4,4'-Diacetoxy-6,6'-dichloro-3,3'-dimethoxychalcone
x——x 6,6'-Dichloro-4,4'-dihydroxy-3,3'-dimethoxychalcone
Specific extinction
Wavelength, mmu
0
20
40
60
80
100
200
220
240
260
280
300
320
340
360
380

DISCUSSION

The isolation of vanillin, 6-chlorovanillin and vanillic acid as oxidation products definitely establishes that chlorite lignin is built around the guaiacyl nucleus, as is the lignin from which it was derived. However, the relatively low yields of identified oxidation products (a maximum of 11% based on chlorite lignin or 1.9% based on the Klason lignin content of the original wood), as well as the low over-all yield of ether-soluble materials, precludes much conjecture on the structure of chlorite lignin or of lignin itself.

The isolation of 6-chlorovanillin and the absence of any detectable quantity of 5-chlorovanillin substantiates the work of Lautsch and Piazolo (15), who found corresponding results with bromolignin.

At first consideration, the lack of any evidence for chlorination in the 5-position is surprising, inasmuch as the presence of free phenolic hydroxyl groups in lignin is well established (38); this group has an ortho-para directing influence and a high activating strength and should induce 5-substitution. However, Barton found the phenolic group to be absent from chlorite lignin. This suggests that the guaiacyl group of lignin, which contains the free phenolic hydroxyl group, may possibly undergo chlorination in the 5-position during the chloriting reaction but that it is cleaved or degraded from the more resistant portion of the lignin molecule which is later isolated as chlorite lignin.

Another interesting speculation to explain the absence of 5-chlorovanillin lies in the known carbon-to-carbon linkage at the 5-position in a

portion of the lignin molecule as evidenced by the isolation of 5-formyl and 5-carboxy compounds; Leopold (39) believed that as much as one half of the guaiacyl nuclei is so substituted. If the nucleus containing the free phenolic group also contains the carbon-to-carbon linkage at the 5-position, no 5-chloro compound could be formed.

Another interesting result, the significance of which is minimized by the low yield of identified products, is the difference between the ratio of chlorinated to unchlorinated compounds in the aldehyde and acid fractions; that is, approximately two thirds as much 6-chlorovanillin as vanillin was found (Oxidation 4), whereas the amount of 6-chlorovanillic acid was insignificant relative to vanillic acid. This suggests that structural factors which favor oxidation of the side chain directly to the carboxyl group discourage chlorine substitution or, conversely, the presence of chlorine in the nucleus stabilizes the molecule at the aldehyde stage of side-chain oxidation.

Similarities in analytical data and oxidation products strongly suggest that the chlorite lignin preparations of Barton and Bublitz are very similar to the material isolated by Wacek and Schroth (5), despite the differences in isolation technique. Barton and Bublitz recovered their products from the chlorite liquor, whereas Wacek and Schroth found their substance to be retained with the holocellulose from which it could be removed by a treatment with 24% potassium hydroxide.

Wacek and Schroth reported that an alkaline nitrobenzene oxidation of their product obtained from an angiosperm produced vanillin, syringaldehyde,

and syringic acid in a combined yield of 19% (based on the weight of the substance). However, the aldehydes were isolated only as the total m-nitrobenzoylhydrazone precipitate. Thus, any chlorinated product was not detected. It seems likely that the application of a chromatographic technique might well have shown the presence of chlorovanillin and/or chlorosyringaldehyde as oxidation products.

The substance which Wacek and Schroth obtained from spruce was not oxidized with alkali and nitrobenzene but with peracetic acid and nickel acetate. They reported the isolation of vanillic acid but gave no details as to technique or quantities found. Again, it is conceivable that, had their lignin preparation been treated with alkali and nitrobenzene and the products separated chromatographically, there may well have been 6-chlorovanillin among the products.

Wacek and Schroth stated, without giving actual values, that the alkaline nitrobenzene oxidation of their angiosperm product yielded considerably more acid than aldehyde. This behavior of chlorite lignin is confirmed by the results found in this work. The amount of vanillic acid formed was approximately equal to the combined yields of vanillin and 6-chlorovanillin. This is to be contrasted with the behavior of wood or other isolated lignins which yield acid and aldehyde in the approximate ratio of 1 to 5. This evidence, with the high carboxyl content of chlorite lignin found by Barton and Wacek and Schroth, clearly shows the partially oxidized character of the preparation.

In view of the small conversion of the formyl group in vanillin to the carboxyl group during oxidation, it may be concluded that a portion of the lignin molecule is susceptible to direct conversion to the carboxyl group; furthermore, the relatively higher yields of vanillic acid from chlorite lignin indicate that chloriting modifies the lignin in such a way as to accentuate this carboxyl group formation. This work provided no evidence to prove or disprove the hypothesis that chlorite lignin contains guaiacyl nuclei in which the side chain has been completely oxidized to the carboxyl group.

The demethylation of vanillin and 6-chlorovanillin in Oxidations 5 and 6 is a phenomenon which had not been observed prior to the advent of chromatographic analysis in the investigation of these products. The low yield of the dihydroxy compounds evidently prevented their detection in earlier work using less sensitive separation methods. The actual yields of the demethylated products were not determined; however, Zentner (40), using chromatographic methods, isolated 2 to 3% of protocatechualdehyde from the reaction of vanillin with a sodium hydrosulfide liquor at 160°. Apparently, the demethylation may be attributed to the alkaline conditions common to both investigations.

Although Hönig and Ruziczka (41) reported that vanillin was quantitatively recovered from a 3-hour treatment with a 10% potassium hydroxide solution at 160°, their determination was based on the m-nitrobenzoylhydrazone precipitate which would not have detected any demethylated product. Stone and Blundell (42) reported a 98% recovery of vanillin from a

micro-scale alkaline nitrobenzene oxidation of the pure compound; the vanillin-containing zone of a paper partition chromatogram was eluted and analyzed spectrophotometrically. It would be of interest to extend their work to include the quantitative estimation of the demethylated and oxidized by-products, now known to be formed, as well as the vanillin itself.

The isolation of 6-chlorovanillin by Pearl (17) from the chlorite liquor itself indicated that degradative oxidation accompanies the formation of chlorite lignin during the chloriting process. Therefore, it is conceivable that the action of nitrobenzene is not particularly suitable for the partially oxidized lignin product but that an entirely different oxidizing agent may be required. Any future work in this field should include a study of agents such as copper oxide or silver oxide, the oxidation potentials of which may be better suited for this application.

SUMMARY AND CONCLUSIONS

1. The alkaline nitrobenzene oxidation of chlorite lignin from slash pine and black spruce produced vanillin, 6-chlorovanillin, and vanillic acid in yields of approximately 4, 2, and 5%, respectively. Thus, the presence of the guaiacyl nucleus in chlorite lignin was clearly established.

2. An oxidation of vanillin alone showed that the formation of vanillic acid was of the order of 1%; the reaction, therefore, cannot account for the ratio of acid-to-aldehyde actually found in the oxidation of wood or isolated lignins which is of the order of 1 to 5. This oxidation of a portion of the lignin molecule directly to the carboxyl group is accentuated by a preliminary chloriting treatment, inasmuch as the oxidation of chlorite lignin produced an amount of vanillic acid approximately equal to the combined yields of vanillin and 6-chlorovanillin.

3. Alkaline nitrobenzene oxidations performed on vanillin, 6-chlorovanillin, and vanillic acid showed that at least 75 to 85% of the starting material survived the oxidative treatment. The aldehydes were partially converted to the corresponding acids. Demethylation was shown to occur with both aldehydes but not with vanillic acid. About 5% of the chlorine contained in the 6-chlorovanillin was converted to chloride ion.

4. In addition to the known method of separating vanillin from vanillic acid on a chromatographic column containing acid-washed Magnesol, use was made of new techniques whereby vanillic acid was chromatographically separated from a mixture of vanillin and 6-chlorovanillin on a column of powdered

cellulose; a method was also developed whereby this separation could be accomplished on a column of anion-exchange resin. It was likewise found that the use of powdered cellulose chromatographic columns permitted the separation of vanillin from 6-chlorovanillin. Paper partition chromatography was found to be an extremely useful tool in the qualitative identification of the phenolic aldehydes and acids encountered in this work.

5. Chromatographic techniques resulted in the isolation of p-phenylazobenzoic acid from the acid fraction of the oxidation products of chlorite lignin. This compound has not previously been reported as a nitrobenzene reduction product.

6. The following new compounds were synthesized by established techniques:

1. 1-(6-Chloro-4-hydroxy-3-methoxyphenyl)-1-ethanol
2. 5-Chlorovanillin acetate
3. 6-Chlorovanillin acetate
4. 6-Chlorovanillin oxime
5. 6-Chlorovanillonitrile acetate
6. 6-Chlorovanillonitrile

7. The silver oxide oxidation of phenolic aldehydes to their corresponding acids was extended to the synthesis of 6-chlorovanillic acid.

8. Demethylation with anhydrous aluminum bromide was extended to the synthesis of 6-chloroprotocatechualdehyde.

9. The previously unreported formation of a chalcone from the reaction of diazomethane with an aromatic aldehyde was noted in two instances. Vanillin acetate and 6-chlorovanillin acetate reacted with diazomethane to

yield intermediates which, upon treatment with dilute alkaline solutions, formed 4,4'-dihydroxy-3,3'-dimethoxychalcone and 6,6'-dichloro-4,4'-dihydroxy-3,3'-dimethoxychalcone, respectively. Further hydrolytic cleavage of the chalcone gave the corresponding aldehyde and methyl ketone in each case. The chlorinated chalcone yielded a new compound, 6-chloroacetovanillone. The same technique was used to prepare the analogous new compound, 5-chloroacetovanillone.

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